

**Application
for
United States Letters Patent**

To all whom it may concern:

Be it known that
Ilya Trakht et al.

have invented certain new and useful improvements in
NOVEL TUMOR-ASSOCIATED MARKER

of which the following is a full, clear and exact description.

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NOVEL TUMOR-ASSOCIATED MARKER

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The invention disclosed herein was made with Government support under NIH Grant No. _____ from the Department of Health and Human Services. Accordingly, the U.S. Government has certain rights in this invention.

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Throughout this application, various publications are referenced by author and date. Full citations for these publications may be found listed alphabetically at the end of the specification immediately preceding the claims. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art.

Background of the Invention

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The seminal discovery by Kohler and Milstein (Kohler, G. and Milstein, C., 1975) of mouse "hybridomas" capable of secreting specific monoclonal antibodies (mAbs) against predefined antigens ushered in a new era in experimental immunology. Many problems associated with antisera were circumvented. Clonal selection and immortality of hybridoma cell lines assured monoclonality and permanent availability of antibody products. At the clinical level, however, the use of such antibodies is clearly limited by the fact that they are foreign proteins and act as antigens in humans.

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Since the report of Kohler and Milstein (Kohler, G. and Milstein, C., 1975), the production of mouse monoclonal antibodies has become routine. However, the application of xenogenic monoclonal antibodies for in vivo diagnostics and therapy is often associated with undesirable effects such as a human anti-mouse immunoglobulin response. In addition, monoclonal antibodies have great potential as tools for imaging. Moreover, therapeutic treatment has motivated the search for means for the production of human monoclonal antibodies (humAbs) (Levy, R., and Miller RA., 1983).

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However, progress in this area has been hampered by the absence of human myelomas suitable as fusion partners with characteristics similar to those of mouse myeloma cells (Posner MR, et al., 1983). The use of Epstein-Barr virus (EBV) has proved to be quite efficient for human lymphocyte immortalization (Kozbor D, and Roder J., 1981; Casual O, 1986), but has certain limitations such as low antibody secretion rate, poor clonogenicity of antibody-secreting lines, and chromosomal instability requiring frequent subcloning. Undifferentiated human lymphoblastoid cell lines appear more attractive. In contrast to differentiated myeloma cells, these cell lines are readily adapted to culture conditions, though the problems of low yield and unstable secretion remain unresolved (Glassy MC, 1983; Ollson L, et al., 1983). The best potential fusion partners are syngenic myeloma cells with well-developed protein synthesis machinery (Nilsson K. and Ponten J., 1975). However, due to culturing difficulties few lines have been conditioned for in vitro growth and capability to produce viable hybrids (Goldman-Leikin RE, 1989). Existing myelomas have low fusion yield and slow hybrid growth, although monoclonal antibody production is relatively stable (Brodin T, 1983). Genetic instability is a major disadvantage of interspecies hybrids. This is the case, for example, when a mouse myeloma is used as the immortalizing partner. Production of mouse-human cell hybrids is not difficult, and these cells have growth characteristics In vitro similar to those of conventional mouse-mouse hybridomas (Teng NNH, 1983). However, spontaneous elimination of human chromosomes considerably reduces the probability of stable mAb secretion (Weiss MC, and Green H., 1967). In order to improve growth characteristics and stability of human monoclonal antibody production, heterohybrids between mouse myeloma cells and human lymphocyte (Oestberg L, and Pursch E., 1983) as well as heteromyelomas (Kozbor D, et. al., 1984) are used as fusion partners.

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established, and thereby several monoclonal antibodies with specific immunoreactivity towards human breast and prostate cancer were developed. These antibodies reacted both with the human cancer cell lines and with primary tumor tissues. These fully human antibodies have specificity to human cancer cell lines as well as primary cancer tissues. Antigen targets were identified for some of these antibodies. Also developed was a hybridoma fusion system, which allows for capturing human lymph node or peripheral blood lymphocytes secreting specific antibodies to cancer antigens. These fully human antibodies may be used to help identify novel tumor-associated antigens, or may be employed for in vivo diagnostic and immunotherapeutic treatment of cancer.

Potential advantages of human monoclonal antibodies include the possibility of identifying the molecular target of the antibody. Such a target could turn out to be a novel molecule altogether or a known molecule whose association with cancer is novel itself. A few years ago scientists at the Ludwig Institute for Cancer Research developed the SEREX method, which allows the identification of novel tumor-associated antigens through the spontaneous antibodies present in cancer patients' blood. Their task was focused specifically on the identification of novel tumor markers. The present invention focused initially on the development of human monoclonal antibodies capable of differentiating cancerous from normal tissue. The identity of a molecular target was secondary to this mission.

In the present invention, molecular targets for some of the antibodies were identified and shown to be specific only for cancer cells. One of the targets which appeared is the PDZ domain containing protein localized both in cytosol and cell membrane of human breast cancer cells. This protein, called GIPC or TIP-2 (Tax interacting protein clone 2) is involved in vesicle trafficking and formation of protein networks. It has several properties, such as the ability

to bind to RGS-Ga interacting protein, C domain, binding to HTLV-1 oncogene tax and binding both to a-actinin and glucose transporter 1. The precise physiological role of this protein is not known, while it shows a consistent overexpression in breast cancer cells, with negligible if any expression in prostate cancer cells and none in human fibroblasts. Although this protein was described previously (2), its association with cancer was not known. It was also not known that a spontaneous antibody response to this marker occurs in breast cancer patients.

One advantage of the present invention is that establishing the association of TIP-2 with malignant transformation allows application of this antigen/protein as a diagnostic marker, both *in vitro* and *in vivo*, for immunohistopathology analysis as well as for immunochemical testing; This protein may be found in the circulation in cancer patients. This protein could also serve as a molecular target for therapeutic purposes given its specific expression in primary tumors. This protein can also be used as a soluble tumor marker for cancer diagnostic, cancer progression and monitoring of cancer treatment in breast and prostate cancer patients. Since this protein is expressed on the surface of cancer cells, it can be used as a target for the specific antibody-driven delivery of liposomes loaded with drugs, or antibody-conjugated drugs, prodrugs, toxins or inhibitors of cell growth. Proving the relevance of TIP-2 for cell survival, this novel marker can be considered as a candidate for vaccine development for immunotherapy of cancer.

Antibodies to TIP-2 derived from breast cancer patient's lymphocytes can be used as a vector for *in vivo* diagnostic (imaging) and immunotherapy (e.g., for delivery of drug-loaded liposomes, or radioimmune- or immunotoxic conjugates to the tumor site). Fully human monoclonal antibodies to TIP-2 can and will be used to isolate preparative quantities of TIP-2 from breast cancer cells or primary

tumors and to develop high affinity mouse antibodies for the purpose of diagnostic and therapeutic use had their biological value been proven. The present invention also provides a basis for the possible development of specific immunoassays or an immunohistochemistry kit for the detection and measurement of this novel tumor marker.

An advantage of the present invention is that human antibodies directed to TIP-2 can be used as an immunosorbent tool for isolation and further characterization of this protein's chemical structure (amino acid composition, protein sequence, modification).

Another advantage of the present invention is an immunosorbent prepared on the basis of human anti-TIP-2 monoclonal antibodies allows isolation of this antigen and its use for developing mouse monoclonal antibodies of high affinity and specificity which can be used to develop better tools for TIP-2 immunoassay.

Another advantage of the present invention is that, knowing the DNA sequence for TIP-2 and its association with cancer, it becomes possible to screen different tissues, normal as well as cancerous, for the expression of this marker.

Another advantage of the present invention is, since human monoclonal antibodies to TIP-2 are available and there is a strong potential to develop non-human antibodies which are even more efficient for certain diagnostic and therapeutic purposes, it is highly likely that TIP-2 can be used as a potential target for immunotherapy and for in vivo diagnostic (imaging).

Another advantage is that since TIP-2 was identified through naturally developed antibodies in breast cancer patients, its existence supports the hypothesis that this antigen can be immunogenic in humans and hence can be

considered as a starting candidate for the development of an anti-cancer vaccine.

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Summary of the Invention

5 The present invention provides a heteromyeloma cell which does not produce any antibody and is capable of producing a trioma cell which does not produce any antibody when fused with a human lymphoid cell; wherein the trioma cell so produced is capable of producing a tetroma cell which produces a monoclonal antibody having specific binding affinity for an antigen when fused with a second human lymphoid cell and such second human lymphoid cell produces an antibody having specific binding affinity for the antigen, with the proviso that the heteromyeloma cell is not B6B11 (ATCC accession number HB-12481).

15 The present invention further provides a trioma cell which does not produce any antibody obtained by fusing a heteromyeloma cell with a human lymphoid cell.

20 The present invention also provides a tetroma cell capable of producing a monoclonal antibody having specific binding affinity for an antigen, obtained by fusing the above-described trioma cell which does not produce any antibody with a human lymphoid cell capable of producing an antibody having specific binding affinity for the antigen.

25 The present invention additionally provides a monoclonal antibody produced by the above-described tetroma.

30 The present invention further provides a method of generating the above-described trioma cell comprising: (a) fusing a heteromyeloma cell which does not produce any antibody with a human lymphoid cell thereby forming trioma cells; (b) incubating the trioma cells formed in step (a) under conditions permissive for the production of antibody by the trioma cells; and (c) selecting a trioma cell that does not produce any antibody.

35 Still further, the present invention provides a method of generating tetroma cells comprising: (a) fusing the

described trioma cell with a human lymphoid cell, thereby forming tetroma cells; (b) incubating the tetroma cells formed in step (a) under conditions permissive for the production of antibody by the tetroma cells; and (c) selecting a tetroma cell capable of producing a monoclonal antibody.

The present invention also provides a method of producing a monoclonal antibody comprising (a) fusing a lymphoid cell capable of producing antibody with the above-described trioma cell, thereby forming tetroma cells; and (b) incubating the tetroma cell formed in step (a) under conditions permissive for the production of antibody by the tetroma cells; (c) selecting a tetroma cell capable of producing the monoclonal antibody; and (d) culturing the tetroma cell of step (c) so as to produce the monoclonal antibody.

Also, the present invention provides a method of producing a monoclonal antibody specific for an antigen associated with a given condition in a subject comprising: (a) fusing a lymphoid cell capable of producing antibody with the above-described trioma cell, thereby forming tetroma cells; (b) incubating the tetroma cell formed in step (a) under conditions permissive for the production of antibody by the tetroma cells; (c) selecting a tetroma cell producing a monoclonal antibody; (d) contacting the monoclonal antibody of step (c) with (1) a sample from a subject with the given condition or (2) a sample from a subject without the given condition, so as to form a complex between the monoclonal antibody and the sample; (e) detecting any complex formed between the monoclonal antibody and the sample; (f) determining the amount of complex formed in step (e); and (g) comparing the amount of complex determined in step (f) for the sample from the subject with the given condition with amount determined in step (f) for the sample from the subject

without the given condition, a greater amount of complex formation for the sample from the subject with the given condition indicating that a monoclonal antibody specific for an antigen specific for the condition has been produced.

Additionally, the present invention provides a method of identifying an antigen associated with a given condition in a sample comprising: (a) contacting the monoclonal antibody produced by the above-described method with the sample, under conditions permissive for the formation of a complex between the monoclonal antibody and the sample; (b) detecting any complex formed in step (a); and (c) isolating any complex detected in step (b), so as to thereby identify the antigen associated with the condition in the sample.

The present invention additionally provides a method of diagnosing a given condition in a subject comprising: (a) contacting a sample from the subject with a monoclonal antibody produced by the above-described method under conditions permissive for the formation of a complex between the monoclonal antibody and the sample; and (b) detecting the formation of any complex formed between the monoclonal antibody and the sample, detection of complex so formed indicating the presence of an antigen specific for the given condition in the sample, and thus providing a diagnosis of the given condition in the subject.

The present invention further provides a composition comprising a monoclonal antibody described by the method described herein and a suitable carrier.

Further, the present invention also provides a therapeutic composition comprising a therapeutically effective amount of a monoclonal antibody of this invention and a pharmaceutically acceptable carrier.

Also, the present invention further provides a method of treating a given condition in a subject comprising administering to the subject an amount of the above-described therapeutic composition effective to treat the condition in the subject.

The present invention also provides a method of preventing a given condition in a subject comprising administering to the subject an amount of the above-described therapeutic composition effective to prevent the condition in the subject.

The present invention provides a monoclonal antibody which specifically binds and forms a complex with TIP-2 antigen located on the surface of human cancer cells, the TIP-2 antigen being an antigen to which monoclonal antibody 27.B1 specifically binds.

The present invention provides the monoclonal antibody 27.B1 produced by the hybridoma having ATCC Accession No. _____

The present invention provides a hybridoma cell producing the monoclonal antibody of this invention.

The present invention provides a pharmaceutical composition comprising the monoclonal antibody of this invention and a pharmaceutically acceptable carrier.

The present invention provides a vaccine comprising the monoclonal antibody of this invention and a pharmaceutically acceptable carrier.

The present invention provides a monoclonal antibody which specifically binds and forms a complex with TIP-2 antigen located on the surface of human cancer cells, the TIP-2 antigen being an antigen to which monoclonal antibody 27.F7 specifically binds.

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The present invention provides the monoclonal antibody 27.F7 produced by the hybridoma having ATCC Accession No. _____

5 The present invention provides a hybridoma cell producing the monoclonal antibody of this invention.

The present invention provides a pharmaceutical composition comprising the monoclonal antibody of this invention and a
10 pharmaceutically acceptable carrier.

The present invention provides a vaccine comprising the monoclonal antibody of this invention and a
15 pharmaceutically acceptable carrier.

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15 The present invention provides a method of detecting TIP-2 antigen bearing cancer cells in a sample comprising: (a) contacting the sample with an antibody directed to an epitope on TIP-2 antigen, or an Fab fragment of an antibody directed to an epitope on TIP-2 antigen, which epitope is recognized by the antibody or the Fab fragment, said antibody or Fab fragment being detectably labeled, under appropriate conditions to produce an antibody/Fab fragment-antigen complex comprising the detectably labeled antibody or Fab fragment bound to any TIP-2 antigen on the surface of cells in the sample; (b) removing any labeled antibody/Fab fragment not bound in the antibody/Fab fragment-antigen complex formed in step (a); and (c)
25 determining presence of the antibody/Fab fragment-antigen complex by detecting the label of the detectably labeled antibody, presence of antibody/Fab fragment-antigen complex indicating TIP-2 antigen-bearing cancer cells in the sample.
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35 The present invention provides a method of detecting TIP-2 antigen bearing cancer cells in a sample comprising: (a) contacting the sample with an antibody directed to an epitope on TIP-2 antigen, or an Fab fragment of an antibody

directed to an epitope on TIP-2 antigen, which epitope is recognized by the antibody or the Fab fragment under appropriate conditions to produce an antibody/Fab fragment-antigen complex comprising the antibody or Fab fragment bound to any TIP-2 antigen on the surface of cells in the sample; (b) removing any antibody/Fab fragment not bound in the antibody/Fab fragment-antigen complex formed in step (a); (c) contacting the antibody/Fab fragment-antigen complex of step (b) with a second antibody which specifically binds to the antibody/Fab fragment-antigen complex, said second antibody being detectably labeled, under appropriate conditions to permit the second labeled antibody to bind to the antibody/Fab fragment-antigen complex; (d) removing any second labeled antibody not bound to the antibody/Fab fragment-antigen complex product in (c); and (e) determining presence of the antibody/Fab fragment-antigen complex bound to the second labeled antibody by detecting the label of second antibody, presence of antibody/Fab fragment-antigen complex indicating TIP-2 antigen-bearing human cancer cells in the sample.

The present invention provides a method of detecting TIP-2 antigen on the surface of cancer cells in a sample comprising: (a) contacting the sample with a antibody directed to an epitope on TIP-2 antigen or an Fab fragment thereof, which epitope is recognized by monoclonal antibody 27.F7 produced by the hybridoma designated _____, said antibody or Fab fragment thereof being detectably labeled, under appropriate conditions to produce an antibody 27.F7/Fab fragment-TIP-2 antigen complex comprising the detectably labeled antibody bound to any TIP-2 antigen on the surface of cells in the sample; b) removing any labeled antibody/Fab fragment not bound in the antibody 27.F7/Fab fragment-TIP-2 antigen complex formed in step (a); and (c) determining presence of the antibody 27.F7/Fab fragment-TIP-2 antigen complex by detecting the label of the detectably labeled antibody, presence of antibody 27.F7/Fab

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fragment-TIP-2 antigen complex indicating TIP-2 antigen-bearing human cancer cells in the sample.

5 The present invention provides a method of detecting TIP-2 antigen on the surface of cancer cells in a sample comprising: (a) contacting the sample with an antibody directed to an epitope on TIP-2 antigen which epitope is recognized by monoclonal antibody 27.F7 produced by the hybridoma designated _____ or Fab fragment thereof, under
10 appropriate conditions to produce an antibody 27.F7/Fab fragment-TIP-2 antigen complex comprising the antibody bound to any TIP-2 antigen on the surface of cells in the sample;

15 (b) removing any antibody or Fab fragment thereof not bound in the antibody 27.F7/Fab fragment-TIP-2 antigen complex formed in step (a); (c) contacting the antibody 27.F7/Fab fragment-TIP-2 antigen complex of step (b) with a second antibody which specifically binds to the antibody 27.F7/Fab fragment-TIP-2 antigen complex, said second antibody being detectably labeled, under appropriate conditions to permit
20 the second labeled antibody to bind to the antibody 27.F7/Fab fragment-TIP-2 antigen complex; (d) removing any second labeled antibody not bound to the antibody 27.F7/Fab fragment-TIP-2 antigen complex product in (c); and (e)
25 determining presence of the antibody 27.F7/Fab fragment-TIP-2 antigen complex bound to the second labeled antibody by detecting the label of second antibody, presence of antibody 27.F7/Fab fragment-TIP-2 antigen complex indicating TIP-2 antigen-bearing human cancer cells in the
30 sample.

35 The present invention provides a method of detecting TIP-2 antigen on the surface of cancer cells in a sample comprising: (a) contacting the sample with a antibody directed to an epitope on TIP-2 antigen or an Fab fragment thereof, which epitope is recognized by monoclonal antibody 27.B1 produced by the hybridoma designated _____ or Fab fragment thereof, said antibody or Fab fragment thereof

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Antibody
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being detectably labeled, under appropriate conditions to produce an antibody 27.B1/Fab fragment-TIP-2 antigen complex comprising the detectably labeled antibody bound to any TIP-2 antigen on the surface of cells in the sample; (b) removing any labeled antibody not bound in the antibody 27.B1-TIP-2 antigen complex formed in step (a); and (c) determining presence of the antibody 27.B1/Fab fragment-TIP-2 antigen complex by detecting the label of the detectably labeled antibody, presence of antibody 27.B1/Fab fragment-TIP-2 antigen complex indicating TIP-2 antigen-bearing human cancer cells in the sample.

The present invention provides a method of detecting TIP-2 antigen on the surface of cancer cells in a sample comprising: (a) contacting the sample with an antibody directed to an epitope on TIP-2 antigen or an Fab fragment thereof, which epitope is recognized by monoclonal antibody 27.B1 produced by the hybridoma designated _____, or Fab fragment thereof under appropriate conditions to produce an antibody 27.B1/Fab fragment-TIP-2 antigen complex comprising the antibody bound to any TIP-2 antigen on the surface of cells in the sample; (b) removing any antibody/Fab fragment thereof not bound in the antibody 27.B1/Fab fragment-TIP-2 antigen complex formed in step (a); (c) contacting the antibody 27.B1/Fab fragment-TIP-2 antigen complex of step (b) with a second antibody which specifically binds to the antibody 27.B1/Fab fragment-TIP-2 antigen complex, said second antibody being detectably labeled, under appropriate conditions to permit the second labeled antibody to bind to the antibody 27.B1/Fab fragment-TIP-2 antigen complex; (d) removing any second labeled antibody not bound to the antibody 27.B1/Fab fragment-TIP-2 antigen complex product in (c); and (e) determining presence of the antibody 27.B1/Fab fragment-TIP-2 antigen complex bound to the second labeled antibody by detecting the label of second antibody, presence of antibody 27.B1/Fab fragment-TIP-2 antigen complex

indicating TIP-2 antigen-bearing human cancer cells in the sample.

5 The present invention provides a method for diagnosing cancer in a subject by detecting TIP-2 antigen-bearing cancer cells which comprises: (a) obtaining a sample of the subject's peripheral blood; (b) contacting the sample with an antibody directed to an epitope on TIP-2 antigen or an Fab fragment thereof, which epitope is recognized by
10 monoclonal antibody 27.F7 produced by the hybridoma designated _____ or an Fab fragment thereof, said antibody being detectably labeled, under appropriate conditions to produce an antibody 27.F7/Fab fragment-TIP-2 antigen complex comprising the detectably labeled antibody bound to any TIP-2 antigen on the surface of cells in the sample; (c) removing any labeled antibody/Fab fragment not bound in the antibody 27.F7/Fab fragment-TIP-2 antigen complex formed in step (b); and (d) determining presence of the antibody 27.F7/Fab fragment-TIP-2 antigen complex by detecting the label of the detectably labeled antibody, presence of antibody 27.F7/Fab fragment-TIP-2 antigen complex indicating diagnosis of cancer in the subject.

25 The present invention provides a method for diagnosing cancer in a subject by detecting TIP-2 antigen-bearing cancer cells which comprises: (a) obtaining a sample of the subject's peripheral blood; (b) contacting the sample with an antibody directed to an epitope on TIP-2 antigen or Fab fragment thereof, which epitope is recognized by monoclonal
30 antibody 27.F7 produced by the hybridoma designated _____ or Fab fragment thereof, under appropriate conditions to produce an antibody 27.F7/Fab fragment-TIP-2 antigen complex comprising the antibody bound to any TIP-2 antigen on the surface of cells in the sample; (c) removing any
35 antibody/Fab fragment not bound in the antibody 27.F7/Fab fragment-TIP-2 antigen complex formed in step (b); (d) contacting the antibody 27.F7/Fab fragment-TIP-2 antigen complex of step (c) with a second antibody which

specifically binds to the antibody 27.F7/Fab fragment-TIP-2 antigen complex, said second antibody being detectably labeled, under appropriate conditions to permit the second labeled antibody to bind to the antibody 27.F7/Fab fragment-TIP-2 antigen complex; (e) removing any second labeled antibody not bound to the antibody 27.F7/Fab fragment-TIP-2 antigen complex product in (d); and (f) determining presence of the antibody 27.F7/Fab fragment-TIP-2 antigen complex bound to the second labeled antibody by detecting the label of second antibody, presence of antibody 27.F7/Fab fragment-TIP-2 antigen complex indicating diagnosis of cancer in the subject.

The present invention provides a method for diagnosing cancer in a subject by detecting TIP-2 antigen-bearing cancer cells which comprises: (a) obtaining a sample of the subject's peripheral blood; (b) contacting the sample with an antibody directed to an epitope on TIP-2 antigen or an Fab fragment thereof, which epitope is recognized by monoclonal antibody 27.B1 produced by the hybridoma designated _____, said antibody being detectably labeled, under appropriate conditions to produce an antibody 27.B1/Fab fragment-TIP-2 antigen complex comprising the detectably labeled antibody bound to any TIP-2 antigen on the surface of cells in the sample; (c) removing any labeled antibody/Fab fragment not bound in the antibody 27.B1/Fab fragment-TIP-2 antigen complex formed in step (b); and (d) determining presence of the antibody 27.B1/Fab fragment-TIP-2 antigen complex by detecting the label of the detectably labeled antibody, presence of antibody 27.B1/Fab fragment-TIP-2 antigen complex indicating diagnosis of cancer in the subject.

The present invention provides a method for diagnosing cancer in a subject by detecting TIP-2 antigen-bearing cancer cells which comprises: (a) obtaining a sample of the subject's peripheral blood; (b) contacting the sample with an antibody directed to an epitope on TIP-2 antigen or Fab

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fragment thereof, which epitope is recognized by monoclonal antibody 27.B1/Fab fragment produced by the hybridoma designated _____ or Fab fragment thereof, under appropriate conditions to produce an antibody 27.B1/Fab fragment-TIP-2 antigen complex comprising the antibody bound to any TIP-2 antigen on the surface of cells in the sample; (c) removing any antibody/Fab fragment not bound in the antibody 27.B1/Fab fragment-TIP-2 antigen complex formed in step (b); (d) contacting the antibody 27.B1/Fab fragment-TIP-2 antigen complex of step (c) with a second antibody which specifically binds to the antibody 27.B1/Fab fragment-TIP-2 antigen complex, said second antibody being detectably labeled, under appropriate conditions to permit the second labeled antibody to bind to the antibody 27.B1/Fab fragment-TIP-2 antigen complex; (e) removing any second labeled antibody not bound to the antibody 27.B1/Fab fragment-TIP-2 antigen complex product in (d); and (f) determining presence of the antibody 27.B1/Fab fragment-TIP-2 antigen complex bound to the second labeled antibody by detecting the label of second antibody, presence of antibody 27.B1/Fab fragment-TIP-2 antigen complex indicating diagnosis of cancer in the subject.

The present invention provides an in vivo method for diagnosing cancer in a subject by detecting TIP-2 antigen-bearing cancer cells which comprises: (a) administering to the subject an antibody directed to an epitope on TIP-2 antigen or Fab fragment thereof, which epitope is recognized by monoclonal antibody 27.F7 produced by the hybridoma designated _____, said antibody being detectably labeled, under appropriate conditions to bind the antibody to TIP-2 antigen on the surface of any cells in the subject; and (b) determining presence of the detectably labeled antibody 27.F7 bound to the surface of cells in the subject, presence of detectably labeled antibody 27.F7 bound to cells indicating diagnosis of cancer in the subject.

The present invention provides an in vivo method for diagnosing cancer in a subject by detecting TIP-2 antigen-bearing cancer cells which comprises: (a) administering to the subject an antibody directed to an epitope on TIP-2 antigen or Fab fragment thereof, which epitope is recognized by monoclonal antibody 27.B1 produced by the hybridoma designated _____, said antibody/Fab fragment being detectably labeled, under appropriate conditions to bind the antibody to TIP-2 antigen on the surface of any cells in the subject; and (b) determining presence of the detectably labeled antibody/Fab fragment 27.B1 bound to the surface of cells in the subject, presence of detectably labeled antibody 27.F7/Fab fragment bound to cells indicating diagnosis of cancer in the subject.

The present invention provides a method for delivering exogenous material to TIP-2 antigen-bearing cancer cells of a human subject comprising administering to the subject a liposome carrying a conjugate of the exogenous material, wherein antibody 27.B1 or an Fab fragment of 27.B1 is coupled to the outer surface of the liposome to target delivery to the cancer cells.

The present invention provides a method for delivering exogenous material to TIP-2 antigen-bearing cancer cells of a human subject comprising administering to the subject a liposome carrying a conjugate of the exogenous material, wherein an antibody 27.F7 or an Fab fragment of 27.F7 is coupled to the outer surface of the liposome to target delivery to the cancer cells.

The present invention provides a method for treating cancer in a human subject by evoking a specific immune response which comprises administering to the subject a whole TIP-2 antigen protein or a peptide fragment of TIP-2 to the subject.

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fragment thereof, which epitope is recognized by monoclonal antibody 27.F7 produced by the hybridoma designated _____, said antibody/Fab fragment being detectably labeled, under appropriate conditions to produce an antibody 27.F7/Fab fragment-TIP-2 antigen complex comprising the detectably labeled antibody bound to any TIP-2 antigen on the surface of cells in the tissue section; (a) removing any labeled antibody/Fab fragment not bound in the antibody 27.F7/Fab fragment-TIP-2 antigen complex formed in step (a); and (b) determining presence of the antibody 27.F7/Fab fragment-TIP-2 antigen complex by detecting the label of the detectably labeled antibody, presence of antibody 27.F7/Fab fragment-TIP-2 antigen complex indicating TIP-2 antigen-bearing human cancer cells in the sample.

The present invention provides a kit for detecting the presence of TIP-2 antigen-bearing cancer cells in a sample comprising: (a) solid support having a plurality of covalently linked probes which may be the same or different, each probe of which comprises a monoclonal antibody directed to an epitope on TIP-2 antigen or Fab fragment thereof; and (b) a means for determining the presence of monoclonal antibody/Fab fragment-TIP-2 antigen complex.

The present invention provides a method for detecting the presence of TIP-2 antigen in biological fluid comprising: (a) contacting a sample of the biological fluid with a antibody directed to an epitope on TIP-2 antigen or Fab fragment thereof, which epitope is recognized by monoclonal antibody 27.F7 produced by the hybridoma designated _____, said antibody being detectably labeled, under appropriate conditions to produce an antibody 27.F7/Fab fragment-TIP-2 antigen complex comprising the detectably labeled antibody bound to any TIP-2 antigen on the surface of cells in the sample; (c) removing any labeled antibody not bound in the antibody 27.F7/Fab fragment-TIP-2 antigen

complex formed in step (a); and (d) determining presence of the antibody 27.F7/Fab fragment-TIP-2 antigen complex by detecting the label of the detectably labeled antibody, presence of antibody 27.F7/Fab fragment-TIP-2 antigen complex indicating TIP-2 antigen-bearing human cancer cells in the biological fluid.

The present invention provides a method for detecting the presence of TIP-2 antigen in biological fluid comprising: (a) contacting a sample of the biological fluid with a antibody directed to an epitope on TIP-2 antigen or Fab fragment thereof, which epitope is recognized by monoclonal antibody 27.B1 produced by the hybridoma designated _____, said antibody being detectably labeled, under appropriate conditions to produce an antibody 27.B1/Fab fragment-TIP-2 antigen complex comprising the detectably labeled antibody bound to any TIP-2 antigen on the surface of cells in the sample; (c) removing any labeled antibody not bound in the antibody 27.B1/Fab fragment-TIP-2 antigen complex formed in step (a); and (d) determining presence of the antibody 27.B1/Fab fragment-TIP-2 antigen complex by detecting the label of the detectably labeled antibody, presence of antibody 27.B1/Fab fragment-TIP-2 antigen complex indicating TIP-2 antigen-bearing human cancer cells in the biological fluid.

The present invention provides a method for immunohistochemical screening of tissue sections from a tumor sample for the presence of TIP-2 antigen-bearing cancer cells which comprises: (a) contacting the tissue section from the tumor sample with a detectably labeled antibody/Fab fragment directed to an epitope on TIP-2 antigen or Fab fragment thereof, which epitope is recognized by monoclonal antibody 27.B1 produced by the hybridoma designated _____, said antibody being detectably labeled, under appropriate conditions to bind the antibody to TIP-2 antigen on the surface of any cells in the sample; and (b) removing any labeled antibody not

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antibody 27.B1 produced by the hybridoma designated _____, said antibody/Fab fragment being detectably labeled, under appropriate conditions to bind the antibody to TIP-2 antigen on the surface of any cells in the subject; (b) determining presence of detectably labeled antibody 27.B1/Fab fragment bound to the surface of cells in the subject the according to the above-described method for detecting TIP-2 antigen on the surface of cancer cells in a sample; (c) comparing the presence of detectably labeled antibody/Fab fragment 27.B1 bound to cells in step (b) with the presence of detectably labeled antibody 27.B1/Fab fragment bound to cells at (i) diagnosis time or (ii) after treatment, wherein a greater presence of detectably labeled antibody 27.B1/Fab fragment bound to cells in step (b) than at (i) diagnosis time or (ii) after treatment, indicates progression of the cancer in the subject and a lesser presence of detectably labeled antibody 27.B1/Fab fragment bound to cells in step (b) than at (i) diagnosis time or (ii) after treatment indicates regression of the cancer in the subject.

The present invention provides a method for monitoring progression of cancer, wherein cancer cells are TIP-2 antigen-bearing cancer cells, in a subject comprising: (a) administering to a subject diagnosed with cancer an antibody directed to an epitope on TIP-2 antigen or an Fab fragment thereof, which epitope is recognized by monoclonal antibody 27.F7 produced by the hybridoma designated _____, said antibody/Fab fragment being detectably labeled, under appropriate conditions to bind the antibody to TIP-2 antigen on the surface of any cells in the subject; (b) determining quantity of detectably labeled antibody 27.F7/Fab fragment bound to the surface of cells in the subject according to the above-described method for detecting TIP-2 antigen on the surface of cancer cells in a sample; (c) comparing the quantity of detectably labeled antibody 27.F7/Fab fragment bound to cells in step (b) with the presence of detectably labeled antibody 27.F7/Fab

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fragment bound to cells at (i) diagnosis time or (ii) after treatment, wherein a greater quantity of detectably labeled antibody 27.F7/Fab fragment bound to cells in step (b) than at (i) diagnosis time or (ii) after treatment, indicates progression of the cancer in the subject and a lesser quantity of detectably labeled antibody 27.F7/Fab fragment bound to cells in step (b) than at (i) diagnosis time or (ii) after treatment indicates regression of the cancer in the subject.

The present invention provides a method for monitoring progression of cancer, wherein cancer cells are TIP-2 antigen-bearing cancer cells, in a subject comprising: (a) administering to a subject diagnosed with the cancer an antibody directed to an epitope on TIP-2 antigen or an Fab fragment thereof, which epitope is recognized by monoclonal antibody 27.B1 produced by the hybridoma designated _____, said antibody/Fab fragment being detectably labeled, under appropriate conditions to bind the antibody to TIP-2 antigen on the surface of any cells in the subject; (b) determining quantity of detectably labeled antibody 27.B1/Fab fragment bound to the surface of cells in the subject according to the above-described method of -----; (c) comparing the quantity of detectably labeled antibody 27.B1/Fab fragment bound to cells in step (b) with the presence of detectably labeled antibody 27.B1 bound to cells at (i) diagnosis time or (ii) after treatment, wherein a greater quantity of detectably labeled antibody 27.B1/Fab fragment bound to cells in step (b) than at (i) diagnosis time or (ii) after treatment, indicates progression of the cancer in the subject and a lesser quantity of detectably labeled antibody 27.B1/Fab fragment bound to cells in step (b) than at (i) diagnosis time or (ii) after treatment indicates regression of the cancer in the subject.

The present invention provides a method for diagnosing cancer associated with the expression of TIP-2 antigen in

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a human subject which comprises: (a) obtaining mRNA from a sample of the subject's peripheral blood; (b) preparing cDNA from the mRNA from step (a); (c) amplifying DNA encoding TIP-2 antigen present in the cDNA prepared in step (b) by a polymerase chain reaction utilizing at least two oligonucleotide primers, wherein each of the primers specifically hybridizes with DNA encoding TIP-2 antigen, wherein the primers comprise oligonucleotides having a sequence included within the sequence of SEQ ID NO. __; and (d) detecting the presence of any resulting amplified DNA, the presence of such amplified DNA being diagnostic for cancer associated with the expression of TIP-2 antigen.

The present invention provides a method for diagnosing cancer associated with the expression of TIP-2 antigen in a human subject which comprises: (a) obtaining mRNA from a sample of the subject's peripheral blood; (b) preparing cDNA from the mRNA from step (a); (c) amplifying DNA encoding TIP-2 antigen present in the cDNA prepared in step (b) by a polymerase chain reaction utilizing at least two oligonucleotide primers, wherein each of the primers specifically hybridizes with DNA encoding TIP-2 antigen, wherein the primers comprise oligonucleotides having a sequence included within the sequence of SEQ ID NO. __; and (d) determining the amount of any resulting amplified DNA; and (e) comparing the amount of amplified DNA determined in step (d) with previously determined standard amounts of amplified DNA, each standard amount being indicative of a particular stage of cancer associated with the expression of TIP-2 antigen.

The present invention further provides a vaccine comprising a monoclonal antibody produced by the method described herein and a suitable carrier.

5 The present invention further provides a method of treating a condition in a subject comprising administering to the subject an amount of the above-described vaccine effective to bind the antigen associated with the condition, thereby treating the condition in the subject.

Finally, the present invention provides a method of preventing a condition in a subject comprising administering to the subject an amount of the above-described vaccine effective to bind the antigen associated with the condition, thereby preventing the condition in the subject.

1990-1991		1991-1992		1992-1993		1993-1994		1994-1995		1995-1996		1996-1997		1997-1998		1998-1999		1999-2000		2000-2001		2001-2002		2002-2003		2003-2004		2004-2005		2005-2006		2006-2007		2007-2008		2008-2009		2009-2010		2010-2011		2011-2012		2012-2013		2013-2014		2014-2015		2015-2016		2016-2017		2017-2018		2018-2019		2019-2020		2020-2021		2021-2022		2022-2023		2023-2024		2024-2025		2025-2026		2026-2027		2027-2028		2028-2029		2029-2030		2030-2031		2031-2032		2032-2033		2033-2034		2034-2035		2035-2036		2036-2037		2037-2038		2038-2039		2039-2040		2040-2041		2041-2042		2042-2043		2043-2044		2044-2045		2045-2046		2046-2047		2047-2048		2048-2049		2049-2050		2050-2051		2051-2052		2052-2053		2053-2054		2054-2055		2055-2056		2056-2057		2057-2058		2058-2059		2059-2060		2060-2061		2061-2062		2062-2063		2063-2064		2064-2065		2065-2066		2066-2067		2067-2068		2068-2069		2069-2070		2070-2071		2071-2072		2072-2073		2073-2074		2074-2075		2075-2076		2076-2077		2077-2078		2078-2079		2079-2080		2080-2081		2081-2082		2082-2083		2083-2084		2084-2085		2085-2086		2086-2087		2087-2088		2088-2089		2089-2090		2090-2091		2091-2092		2092-2093		2093-2094		2094-2095		2095-2096		2096-2097		2097-2098		2098-2099		2099-2100		2100-2101		2101-2102		2102-2103		2103-2104		2104-2105		2105-2106		2106-2107		2107-2108		2108-2109		2109-2110		2110-2111		2111-2112		2112-2113		2113-2114		2114-2115		2115-2116		2116-2117		2117-2118		2118-2119		2119-2120		2120-2121		2121-2122		2122-2123		2123-2124		2124-2125		2125-2126		2126-2127		2127-2128		2128-2129		2129-2130		2130-2131		2131-2132		2132-2133		2133-2134		2134-2135		2135-2136		2136-2137		2137-2138		2138-2139		2139-2140		2140-2141		2141-2142		2142-2143		2143-2144		2144-2145		2145-2146		2146-2147		2147-2148		2148-2149		2149-2150		2150-2151		2151-2152		2152-2153		2153-2154		2154-2155		2155-2156		2156-2157		2157-2158		2158-2159		2159-2160		2160-2161		2161-2162		2162-2163		2163-2164		2164-2165		2165-2166		2166-2167		2167-2168		2168-2169		2169-2170		2170-2171		2171-2172		2172-2173		2173-2174		2174-2175		2175-2176		2176-2177		2177-2178		2178-2179		2179-2180		2180-2181		2181-2182		2182-2183		2183-2184		2184-2185		2185-2186		2186-2187		2187-2188		2188-2189		2189-2190		2190-2191		2191-2192		2192-2193		2193-2194		2194-2195		2195-2196		2196-2197		2197-2198		2198-2199		2199-2200		2200-2201		2201-2202		2202-2203		2203-2204		2204-2205		2205-2206		2206-2207		2207-2208		2208-2209		2209-2210		2210-2211		2211-2212		2212-2213		2213-2214		2214-2215		2215-2216		2216-2217	
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Brief Description of the Figures

Figures 1A-1C

Distribution of cells according to the number of chromosomes. The X-axis indicates the amount of chromosomes. The Y-axis indicates the percentage of cells with appropriate number of chromosomes. The data represent the average ones based on the analysis of more than 50 metaphase plates for each line: P3.X63.Ag8.653 Fig.1A, RPMI 8226 Fig.1B, B6B11 Fig.1C.

Figure 2

Fragment of G-banded karyotype of B6B11 line. The arrows indicate genetic material presumably of human origin; 3p portion of chromosome 3 and chromosome 19.

Figure 3

B6B11 fusion efficiency with fresh isolated and cultured splenocytes. SPL were isolated in LSM, immediately after a portion of the cells were fused with B6B11 cells and the remaining SPL were cultivated in vitro for 7-9 days in RPMI-C containing 15% FCS in the presence of ConA, LPS, PHA, PWM or without mitogens, then these cells were also fused with B6B11. PWM in the concentration of 5 μ g/ml influenced effectively the fusion efficiency.

Figures 4A-4D

DNA histograms of parental cells 653 (Fig.4A) and 8226 (Fig.4B), heteromyeloma B6B11 (Fig.4C) and B6B11-splenocyte hybrid (Fig.4D). The amount of B6B11 DNA constitutes about 100% of the total amount of 653 DNA plus 8226 DNA. The DNA content of B6B11-SPL hybrid is greater than that of B6B11.

Figures 5A-5B

Immunoglobulin production by hybridomas (tetromas) derived from the fusion of PBLs with MFP-2. **Figure 5A** shows results of fusing fresh lymphocyte suspensions with MFP-2. **Figure 5B** shows results of fusing frozen/thawed lymphocyte suspensions with MFP-2. The dark rectangles indicate IgM production. The gray rectangles indicate IgG production. The Y-axis indicates optical density at A_{490} for different hybridoma samples (tetromas) generated from fusion with the MFP-2 trioma line (X-axis). The dotted line indicates the optical density at A_{490} for a 1:500 dilution of IgM antibody. The dashed line indicates the optical density at A_{490} for a 1:500 dilution of IgG antibody.

Figure 6

Anti-thyroglobulin antibody production by thyroid cancer lymph node lymphocytes fused to fusion partner MFP-2 cells. The Y-axis indicates optical density at A_{405} (OD_{405}) for different hybridoma samples (tetromas) generated from fusion with the MFP-2 trioma line (X-axis). Thirty-three tetromas produced antibody which reacted positively against thyroglobulin; eight were particularly strongly reactive.

Figure 7

Flow cytometry analysis of fixed and live cells treated with anti-TIP-2 fhMAbs. Green = control; Red = cells treated with antibodies.

Figure 8

Western blot analysis of breast and prostate cancer celllysates for the presence of TIP-2. Two non-transformed human fibroblasts cell lines were used as a negative control. Human monoclonal anti-TIP-2 antibodies 27.B1 and 27.F7 were used as a tag. 7 mg of

total cell lysate protein was applied to each line. The strong TIP-2 expression can be observed in breast cancer cells.

5 **Figure 9**

Immunofluorescence staining of formalin-fixed human cells with human monoclonal anti-TIP-2 antibodies 27.B1 and 27.F7. Size bars represent 20 mm. On this and other figures with immunofluorescence staining red is a propidium iodide counterstaining of cell nuclei and green is FITC-labeled antibody staining. Confocal microscopy was done for SK-BR-3 breast cancer cells.

10 **Figure 10**

Immunofluorescence staining of normal and cancerous human breast tissues using human anti-TIP-2 monoclonal antibody 27.B1. Upper panel - different cases of invasive ductal adenocarcinoma; lower panel - normal breast tissue. Size bars represent 20 mm.

15 **Figure 11**

Immunofluorescence staining of human prostate tissues using human anti-TIP-2 monoclonal antibody 27.B1. Upper panel - different cases of prostate adenocarcinoma; lower panel - benign prostate hypertrophy as negative control. Size bars represent 20 mm.

20 **Figure 12**

Same as Fig. 4, but with fhMAB 27.F7.

25 **Figure 13**

Same as Fig. 5, but with fhMAB 27.F7.

30 **Figure 14**

Immunofluorescence staining of lymph nodes with breast cancer metastatic spread. Human monoclonal anti-TIP-2 antibodies 27.B1 and 27.F7 were used in this experiment. Size bars represent 20 mm.

5

Figure 15

Formalin fixed and freshly frozen sections of breast adenocarcinoma using two anti-TIP-2 antibodies 27.B1 and 27.F7. Size bars represent 20 mm.

10

Figure 16

Immunofluorescence staining of male breast intraductal carcinoma and seminoma using fhMAbs 27.F7 and 27.B1. Size bars represent 20 mm.

15

Figure 17

Immunofluorescence staining of breast cancer and other cancerous and normal tissues using fhMAbs 27.F7 and 27.B1. Size bars represent 20 mm.

20

Figure 18

Schematic view of G-protein signaling system

Figure 19

Regulators of G-signaling system and PDZ domain-containing proteins.

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Figure 20

Principle of SEREX technology

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Figure 21

Immunization of mice to TIP-2 using immunoprecipitation with human anti-TIP-2 antibody and Western blotting.

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The release of TIP-2 antigen into cell culture media of SK-BR-3 cells treated with different concentration of Taxol. The lines are as follows (from left to right): 1) SK-BR-3 cell lysate prepared from approximately 70,000 cells; 2) empty lane; 3) Taxol, 88uM added to 35 mm tissue plate containing approximately 250,000 cells; 4) same with Taxol, 44uM; 5) same with Taxol, 22uM; 7) same with Taxol, 11uM; 8) same with Taxol, 5.5uM; 9) cell lysate prepared from cells which were not treated with Taxol; 10) lysate

prepared from the residual dead cells' remnants after treatment with Taxol, 88uM.

1 Figure 29

The amino acid sequence of GIPC/TIP-2 protein. In italics, the amino acid sequence of TIP-2 only. Underlined are two peptides identified as high HLA-*A0201 binders (theoretical calculation).

Figure 30

The mRNA sequence of GIPC. The part of the sequence corresponding to TIP-2 is underlined.

Figure 31

Protein Antigens Identified by Natural Human Monoclonal Antibodies Developed from Breast and Prostate Cancer Patients' B-Cells.

Figure 32

Human mRNA sequence for KIAA0338 gene, partial cds.

Figure 33

Human non-muscle alpha-actinin mRNA sequence, complete cds - the second non-muscle alpha-actinin isoform designated ACTN4 (actinin-4).

Figure 34

Homo sapiens actinin, alpha 4 (ACTN4) mRNA sequence.

Figure 35

Clathrin coat assembly protein AP50 mRNA sequence.

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Figure 36

Homo sapiens GLUT1 C-terminal Binding protein
(GLUT1CBP) mRNA sequence.

10

Figure 37

gp130 associated protein GAM sequence.

Figure 38

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Homo sapiens amino-terminal enhancer of split (AES)
mRNA sequence.

Figure 39

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Antiquitin 1 (antiquitin=26g turgor protein homolog),
mRNA sequence.

Figure 40

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ARP2/3 protein complex 41 KD subunit (P41-ARC), mRNA
sequence.

Figure 41a

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H. sapiens seb4D mRNA sequence.

Figure 41b

35

H. sapiens seb4B mRNA sequence.

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Figure 42

Homo sapiens lamin A/C (LMNA) mRNA sequence.

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Detailed Description of the Invention

5 The present invention provides a heteromyeloma cell which does not produce any antibody and is capable of producing a trioma cell which does not produce any antibody when fused with a human lymphoid cell; wherein the trioma cell so produced is capable of producing a tetroma cell which produces a monoclonal antibody having specific binding affinity for an antigen when fused with a second human lymphoid cell and such second human lymphoid cell of
10 produces an antibody having specific binding affinity for the antigen, with the proviso that the heteromyeloma cell is not B6B11 (ATCC accession number HB-12481).

15 The present invention also provides a trioma cell which does not produce any antibody obtained by fusing a heteromyeloma cell with a human lymphoid cell. In one embodiment of this invention, the heteromyeloma cell is the cell designated B6B11 (ATCC accession number HB-12481). In another embodiment, the trioma is a B6B11-like cell. For
20 purposes of this invention a B6B11-like cell includes a cell which is substantially identical to the B6B11 cell at the genetic level and a functionally equivalent thereto. B6B11-like cells thus specifically include clones or other cells derived from B6B11 including mutants of the B6B11 and
25 of clones thereof. In certain embodiments of this invention, the human lymphoid cell is a myeloma cell. In other embodiments of this invention, the human lymphoid cell is a splenocyte or a lymph node cell (lymphocyte). According to certain embodiments of this invention, the
30 trioma cell is the cell designated MFP-2 (ATCC accession number 12482).

35 The present invention also provides a tetroma cell capable of producing a monoclonal antibody having specific binding affinity for an antigen, obtained by fusing the above-described trioma cell which does not produce any antibody with a human lymphoid cell capable of producing antibody having specific binding affinity for the antigen. The

human lymphoid cell may be a peripheral blood lymphocyte, a splenocyte, a lymph node cell, a B cell, a T cell, a tonsil gland lymphocyte, a monocyte, a macrophage, an erythroblastoid cell or a Peyer's patch cell. In one embodiment of this invention, the trioma cell is the cell designated MFP-2 (ATCC accession number HB-12482).

According to certain embodiments of this invention, the antigen is a tumor-associated antigen, a cell-specific antigen, a tissue-specific antigen, an enzyme, a nucleic acid, an immunoglobulin, a toxin, a viral antigen, a bacterial antigen or a eukaryotic antigen. In one embodiment, the antigen is a mammalian, insect, fungal, E.coli or Klebsiella antigen.

The present invention provides a monoclonal antibody produced by the above-described tetroma. The present invention also provides an isolated nucleic acid encoding the monoclonal antibody produced by the described tetroma. The nucleic acid may include, but is not limited to DNA, RNA, cDNA, oligonucleotide analogs, vectors, expression vectors or probes. Additionally, the present invention contemplates the expression of the nucleic acid encoding the monoclonal antibody introduced into a host cell capable of expression the monoclonal antibody or portions thereof.

The present invention also provides isolated nucleic acids including all or a portion of the antibody binding regions of such monoclonal antibodies and the use of such nucleic acid to express portions of such antibodies, for example, single chain antibodies per se or phage-displayed single chain antibodies (sFv-a antibody).

Moreover, nucleic acids encoding all or a portion of such nucleic acids may be used to transfect mammalian cells such as mouse myeloma or CHO cells to permit increased production of such monoclonal antibody or portion thereof.

The present invention further provides a method of generating the described trioma cell comprising: (a) fusing a heteromyeloma cell which does not produce any antibody with a human lymphoid cell thereby forming trioma cells;
5 (b) incubating the trioma cells formed in step (a) under conditions permissive for the production of antibody by the trioma cells; and (c) selecting a trioma cell that does not produce any antibody.

10 According to one embodiment of this invention, the heteromyeloma cell of step (a) is designated B6B11 (ATCC accession number HB-12481). According to other embodiments of this invention, the human lymphoid cell is a lymph node lymphocyte or a splenocyte. According to certain
15 embodiments of the present invention, the method further comprises selecting a trioma cell capable of growth in serum-free media. Other embodiments comprise selecting a trioma cell that is capable of fusing with a peripheral blood lymphocyte or lymph node lymphocyte. The present
20 invention further provides a trioma cell generated by the above-described method.

Still further, the present invention provides a method of generating a tetroma cell comprising: (a) fusing the above-
25 described trioma cell with a human lymphoid cell thereby forming tetroma cells; (b) incubating the tetroma cell formed in step (a) under conditions permissive to the production of antibody by the tetroma cells; and (c) selecting a tetroma cell capable of producing a monoclonal
30 antibody. According to one embodiment of this invention, the trioma cell of step (a) the cell is designated MFP-2 (ATCC accession number HB-12482). According to an embodiment of this invention, the human lymphoid cell is a peripheral blood lymphocyte, a splenocyte, a lymph node
35 cell, a B cell, a T cell, a tonsil gland lymphocyte, a monocyte, a macrophage, an erythroblastoid cell or a Peyer's patch cell. In some embodiments of this invention, the human lymphoid cell produces antibodies having specific

binding affinity for an antigen and the tetroma cell produces a monoclonal antibody having specific binding affinity for such antigen. According to certain embodiments of this invention, the antigen is a tumor-associated antigen, a cell-specific antigen, a tissue-specific antigen, an enzyme, a nucleic acid, an immunoglobulin, a toxin, a viral antigen, a bacterial antigen, or a eukaryotic antigen. In some embodiments of this invention, the antigen is a mammalian, insect, E.coli or Klebsiella antigen. The present invention further provides a tetroma cell generated by the above-described method.

This invention also provides human hybridoma fusion partner cell line heteromyeloma B6B11, and human hybridoma fusion partner cell line trioma MFP-2. These hybridoma cell lines were deposited on March 17, 1998 with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.S. under the provision of the Budapest Treaty for the International Recognition of the Deposit of Microorganism for the Purposes of Patent Procedure. These hybridoma have been accorded with ATCC Accession Nos. HB-12481 and HB-12482 respectively.

The present invention also provides a method of producing a monoclonal antibody comprising (a) fusing a lymphoid cell capable of producing antibody with the described trioma cell, thereby forming a tetroma cell; and (b) incubating the tetroma cell formed in step (a) under conditions permissive for the production of antibody by the tetroma cell so as to thereby produce the monoclonal antibody.

Also, the present invention provides a method of producing a monoclonal antibody specific for an antigen associated with a given condition in a subject comprising: (a) fusing a lymphoid cell capable of producing antibody with the above-described trioma cell, thereby forming tetroma cells; (b) incubating the tetroma cell formed in step (a) under

conditions permissive for the production of antibody by the tetroma cells; (c) selecting a tetroma cell producing a monoclonal antibody; (d) contacting the monoclonal antibody of step (c) with (1) a sample from a subject with the given condition or (2) a sample from a subject without the given condition under conditions permissive to the formation of a complex between the monoclonal antibody and the sample; (e) detecting the complex formed between the monoclonal antibody and the sample; (f) determining the amount of complex formed in step (e); and (g) comparing the amount of complex determined in step (f) for the sample from the subject with the condition with amount determined in step (f) for the sample from the subject without the condition, a greater amount of complex formation for the sample from the subject with the condition indicating that a monoclonal antibody specific for the antigen specific for the condition has been produced.

In one embodiment of the present invention, step (a) further comprises freezing the lymphoid cell. According to one embodiment of the present invention, step (c) further comprises incubating the selected tetroma cell under conditions permissive for cell replication. According to certain embodiments of this invention, the tetroma replication is effected in vitro or in vivo. According to one embodiment of this invention, the trioma cell is the cell designated MFP-2 (ATCC Accession No. HB-12482). The present invention provides a monoclonal antibody specific for an antigen associated with a condition, identified by the described method. The present invention also provides an isolated nucleic acid encoding the described monoclonal antibody. The nucleic acid may include, but is not limited to DNA, RNA, cDNA, oligonucleotide analogs, vectors, expression vectors or probes. Additionally, the present invention contemplates the expression of the nucleic acid encoding the monoclonal antibody introduced into a host

cell capable of expression the monoclonal antibody or portions thereof.

5 The present invention also provides isolated nucleic acids including all or a portion of the antibody binding regions of such monoclonal antibodies and the use of such nucleic acid to express portions of such antibodies, for example, single chain antibodies per se or phage-displayed single chain antibodies (sFv-a antibody).

10 Moreover, nucleic acids encoding all or a portion of such nucleic acids may be used to transfect mammalian cells such as mouse myeloma or CHO cells to permit increased production of such monoclonal antibody or portion thereof.

15 According to an embodiment of this invention, the given condition as is associated with a cancer, a tumor, a toxin, an infectious agent, an enzyme dysfunction, a hormone dysfunction, an autoimmune disease, an immune dysfunction, 20 a viral antigen, a bacterial antigen, a eukaryotic antigen, rejection of a transplanted tissue, poisoning, or venom intoxication. Additionally, the condition may be any other abnormality, including that resulting from infection, cancer, autoimmune dysfunction, cardiovascular disease, or 25 transplantation. In an embodiment of this invention, the given condition is septicemia, sepsis, septic shock, viremia, bacteremia or fungemia. In certain embodiments of this invention, the cancer may be, but is not limited to lung cancer, liver cancer, leukemia, lymphoma, 30 neuroblastoma, glioma, meningioma, bone cancer, thyroid cancer, ovarian cancer, bladder cancer, pancreatic cancer, breast cancer, or prostate cancer. According to certain embodiments of this invention, the infectious agent may be, but is not limited to Hanta virus, HTLV I, HTLV II, HIV, 35 herpes virus, influenza virus, Ebola virus, human papilloma virus, Staphylococcus, Streptococcus, Klebsiella, E. coli, anthrax, or cryptococcus. According to certain embodiments of this invention, the toxin is tetanus, anthrax, botulinum

snake venom or spider venom. In one embodiment of this invention, the tumor is benign. In another embodiment, the enzyme dysfunction is hyperactivity or overproduction of the enzyme. In still another embodiment, the hormone dysfunction is hyperactivity or overproduction of the hormone. In yet other embodiments of this invention, the immune dysfunction is CD3 or CD4 mediated. In still other embodiments of this invention, the autoimmune disease is lupus, thyroidosis, graft versus host disease, transplantation rejection, or rheumatoid arthritis. In still other embodiments of the invention, the condition is any abnormality. In still other embodiments, the condition is the normal condition.

Additionally, the present invention provides a method of identifying an antigen associated with a given condition in a sample comprising: (a) contacting the monoclonal antibody produced by the above-described method with the sample under conditions permissive for the formation of a complex between the monoclonal antibody and the sample; (b) detecting any complex formed in step (a); and (c) isolating the complex detected in step (b), thereby identifying the antigen associated with the condition in the sample.

In one embodiment of the above-described method, the condition is a tumor.

In another embodiment of the above-identified method, the antigen is not previously known.

This invention also provides a tumor antigen identified by the above-described method where the antigen is not previously known.

This invention also provides a method for diagnosing a tumor in a sample comprising detecting the presence of the tumor antigen identified by the above-described method

wherein the condition is a tumor, the presence of said antigen indicating the presence of tumor in the subject.

5 This invention also provides the above-described method, wherein the detecting comprises: (a) obtaining an appropriate sample which contains the tumor antigen from the subject; (b) contacting the sample with an antibody which is capable of specifically binding to the tumor antigen under conditions permitting the formation of a complex
10 between the antibody and the antigen; and (c) detecting the complex formed, thereby detecting the presence of the tumor antigen.

15 In certain embodiments of this invention, the method further comprises separating the monoclonal antibody from the monoclonal antibody-antigen complex. In some embodiments the separation is by size fractionation, e.g. the size fractionation effected by polyacrylamide or agarose gel electrophoresis.

20 According to certain embodiments of this invention, the given condition is associated with, a cancer, a tumor, a toxin, an infectious agent, an enzyme dysfunction, a hormone dysfunction, an autoimmune disease, an immune dysfunction, a viral antigen, a bacterial antigen, a eukaryotic antigen, rejection of a transplanted tissue, poisoning, or venom intoxication. Additionally, the condition may be any other abnormality, including one
25 resulting from infection, cancer, autoimmune dysfunction, cardiovascular disease, or transplantation. In an embodiment of this invention, the condition is septicemia, sepsis, septic shock, viremia, bacteremia or fungemia. In some embodiments of this invention, the cancer may be but is not limited to lung cancer, liver cancer, leukemia, lymphoma, neuroblastoma, glioma, meningioma, bone cancer, thyroid cancer, colon cancer, ovarian cancer, bladder
30 cancer, pancreatic cancer, breast cancer or prostate cancer. According to some embodiments of this invention,

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the infectious agent may be but is not limited to Hanta virus, HTLV I, HTLV II, HIV, herpes virus, influenza virus, Ebola virus, human papilloma virus, Staphlococcus, Streptococcus, Klebsiella, E. coli, anthrax or cryptococcus. According to some embodiments of this invention, the toxin is tetanus, anthrax, botulinum, snake venom or spider venom. In one embodiment of this invention, the tumor is benign. In other embodiments, the enzyme dysfunction is hyperactivity or overproduction of the enzyme. In still other embodiments, the hormone dysfunction is hyperactivity or overproduction of the hormone. In yet other embodiments of this invention, the immune dysfunction is CD3 or CD4 mediated. In still other embodiments of this invention, the autoimmune disease is lupus, thyroidosis, graft versus host disease, transplantation rejection or rheumatoid arthritis. In still other embodiments of the invention, the condition is any abnormality. In still other embodiments, the condition is the normal condition.

The present invention additionally provides a method of diagnosing a condition in a subject comprising: (a) contacting a sample from the subject with a monoclonal antibody produced by the above-described method under conditions permissive for the formation of a complex between the monoclonal antibody and the sample; and (b) detecting the formation of any complex formed between the monoclonal antibody and the sample, positive detection of such complex indicating the presence of an antigen specific for the condition in the sample which correlates with diagnosing the condition in the subject.

According to an embodiment of this invention, the monoclonal antibody is coupled to a detectable marker. In an embodiment of this invention, the detectable marker is a radiolabel, a fluorophore, or fluorescent molecule, an

enzyme, a ligand, a colorimetric marker, or a magnetic bead.

According to some embodiments of this invention, the given condition is or is associated with, a cancer, a tumor, a toxin, an infectious agent, an enzyme dysfunction, a hormone dysfunction, an autoimmune disease, an immune dysfunction, a viral antigen, a bacterial antigen, a eukaryotic antigen, rejection of a transplanted tissue, poisoning, or venom intoxication. Additionally the condition may be any other abnormality, including one resulting from infection, cancer, autoimmune dysfunction, cardiovascular disease, or transplantation. In certain embodiments of this invention, the condition is septicemia, sepsis, septic shock, viremia, bacteremia or fungemia. In some embodiments of this invention, the cancer may be, but is not limited to lung cancer, liver cancer, leukemia, lymphoma, neuroblastoma, glioma, meningioma, bone cancer, thyroid cancer, ovarian cancer, bladder cancer, pancreatic cancer, breast cancer or prostate cancer. According to other embodiments of this invention, the infectious agent may be, but is not limited to Hanta virus, HTLV I, HTLV II, HIV, herpes virus, influenza virus, Ebola virus, human papilloma virus, Staphylococcus, Streptococcus, Klebsiella, E. coli, anthrax or cryptococcus. According to some embodiments of this invention, the toxin is tetanus, anthrax, botulinum, snake venom or spider venom. In one embodiment of this invention, the tumor is benign. In other embodiments, the enzyme dysfunction is hyperactivity or overproduction of the enzyme. In still other embodiments, the hormone dysfunction is hyperactivity or overproduction of the hormone. In yet other embodiments of this invention, the immune dysfunction is CD3 or CD4 mediated. In still other embodiments of this invention, the autoimmune disease is lupus, thyroiditis, graft versus host disease, transplantation rejection or rheumatoid arthritis. In still other embodiments of the invention,

the condition is any abnormality. In still other embodiments, the condition is the normal condition.

5 The present invention further provides a composition comprising a monoclonal antibody produced by the method described herein and a suitable carrier.

10 Further, the present invention also provides a therapeutic composition comprising a therapeutically effective amount of a monoclonal antibody of this invention and a pharmaceutically acceptable carrier.

15 According to certain embodiments of this invention, the condition is cancer and the amount of monoclonal antibody is sufficient to inhibit the growth of or eliminate the cancer. According to certain embodiments, the condition is an infection and the amount of monoclonal antibody is sufficient to inhibit the growth of or kill the infectious agent. According to certain embodiments of this invention, 20 the condition is associate with a toxin and the amount of monoclonal antibody is sufficient to reduce the amount of or destroy the toxin. In still other embodiments, the condition is an autoimmune disease and the amount of monoclonal antibody is sufficient to reduce the amount of or destroy the offending antibody or subunit(s) thereof. 25 In still other embodiments, the condition is a cardiovascular disease and the amount of monoclonal antibody is sufficient to reduce the condition. In yet other embodiments, the condition is a transplantation rejection, and the amount of monoclonal antibody is 30 sufficient to reduce the condition.

35 According to certain embodiments of this invention, the monoclonal antibody is coupled to an effector compound. In certain embodiments of this invention, the effector compound is a cytotoxic agent, drug, enzyme, dye, or radioisotope. In certain embodiments of this invention,

invention, the tumor is benign. In another embodiment, the enzyme dysfunction is hyperactivity or overproduction of the enzyme. In still another embodiment, the hormone dysfunction is hyperactivity or overproduction of the hormone. In yet other embodiments of this invention, the immune dysfunction is CD3 or CD4 mediated. In still other embodiments of this invention, the autoimmune disease is lupus, thyroidosis, graft versus host disease, transplantation rejection or rheumatoid arthritis. In still other embodiments of the invention, the condition is any abnormality. In still other embodiments, the condition is the normal condition.

Finally, the present invention provides a method of preventing a given condition in a subject comprising administering to the subject an amount of the above-described therapeutic composition effective to prevent the condition in the subject. In one embodiment of this invention, the subject previously exhibited the condition. According to one embodiment of this invention, the therapeutic composition is administered to a second subject.

According to certain embodiments of this invention, the condition is or is associated with a cancer, a tumor, a toxin, an infectious agent, an enzyme dysfunction, a hormone dysfunction, an autoimmune disease, an immune dysfunction, a viral antigen, a bacterial antigen, a eukaryotic antigen, rejection of a transplanted tissue, poisoning, or venom intoxication. Additionally, the condition may be any other abnormality, including one resulting from infection, cancer, autoimmune dysfunction, cardiovascular disease, or transplantation. In certain embodiments of this invention, the condition is septicemia, sepsis, septic shock, viremia, bacteremia or fungemia. In some embodiments of this invention, the cancer may be but is not limited to lung cancer, liver cancer, leukemia,

lymphoma, neuroblastoma, glioma, meningioma, bone cancer, thyroid cancer, colon cancer, ovarian cancer, bladder cancer, pancreatic cancer, breast cancer or prostate cancer. According to an embodiment of this invention, the infectious agent may be but is not limited to Hanta virus, HTLV I, HTLV II, HIV, herpes virus, influenza virus, Ebola virus, human papilloma virus, Staphylococcus, Streptococcus, Klebsiella, E. coli, anthrax or cryptococcus. According to some embodiments of this invention, the toxin is tetanus, anthrax, botulinum, snake venom or spider venom. In one embodiment of this invention, the tumor is benign. In other embodiments, the enzyme dysfunction is hyperactivity or overproduction of the enzyme. In still other embodiments, the hormone dysfunction is hyperactivity or overproduction of the hormone. In yet other embodiments of this invention, the immune dysfunction is CD3 or CD4 mediated. In still other embodiments of this invention, the autoimmune disease is lupus, thyroidosis, graft versus host disease, transplantation rejection or rheumatoid arthritis. In still other embodiments of the invention, the condition is any abnormality. In still other embodiments, the condition is the normal condition.

The present invention also provides the production of antibodies for antigens which are not associated with a given condition, but more properly constitute a component of the entire repertoire of antibodies in a human immune system.

In addition, the present invention provides identification of novel antigens relevant to a given condition in a subject and the use thereof for diagnosis and treatment of the given condition in the subject. The invention also provides identification of the repertoire of naturally occurring antibodies in normal subjects and subjects having a pathological condition. In one embodiment, the condition may be venom detoxification (neutralization). For

example, the condition may result from scorpion, spider, rattle snake or poison toad bites or venom exposure. The present invention provides antibodies to act as antidote for such conditions.

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The trioma cell of the present invention may also be fused with macrophages, monocytes, T-lymphocytes, and erythroblastoid cells. Hybridoma cells resulting from such fusions may produce growth factors, cytokines, enzymes, hemoglobin .

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As used herein, a human-murine hybridoma (the "immortalizing hybridoma") is an immortal cell line which results from the fusion of a murine myeloma or other murine tumor cell with a human lymphoid cell derived from a normal subject. As described herein below, by careful selection and mutation, an immortalizing hybridoma which provides improved chromosomal stability, has human characteristics, and which does not secrete immunoglobulin may be obtained. The antibody secreting capability of such a resulting trioma may be provided by the third cell fusion which is typically derived either from B cells of an immunized human individual, or with B cells which have been immortalized.

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As used herein, a "B6B11" cell is a hybrid cell produced by the fusion of mouse myeloma 653 and human myeloma RPMI 8226.

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As used herein, a "B6B11-like" cell is a a hybrid cell produced by the fusion of mouse myeloma 653-related cell and human myeloma RPMI 8226-related cell.

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As used herein, a "MFP" cell is a hybrid cell produced by the fusion of a B6B11 cell and a human lymphocyte. B6B11-like cells share function properties and characteristics with B6B11 heteromyeloma cells.

As used herein, a "MFP-like" cell is a hybrid cell produced by the fusion of a B6B11-like cell and a human lymphocyte. MFP-like cells share function properties and characteristics with MFP trioma cells.

As used herein, "non-secreting" or "non-producing" hybridoma refers to a hybridoma which is capable of continuous reproduction and, therefore, is immortal, and which does not produce immunoglobulin.

As used herein, a hybridoma "having human characteristics" refers to a hybridoma which retains detectable human-derived chromosomes such as those producing human HLA antigen which may be expressed on the cell surface.

As used herein, lymphoid cells "immunized against a predefined determinant" refers to lymphoid cells derived from an subject who has been exposed to an antigen having the determinant. For example, a subject can be induced to produce (from its lymphoid B cells) antibodies against the antigenic determinants of various blood types, by exposure, through transfusions or previous pregnancy, or against the antigenic determinants of specific viruses or of bacteria by virus of exposure through past infections or vaccinations.

As used herein, "cell line" refers to various embodiments including but not limited to individual cells, harvested cells and cultures containing cells so long as these are derived from cells of the cell line referred to may not be precisely identical to the ancestral cells or cultures and any cell line referred to include such variants.

As used herein, "trioma" refers to a cell line which contains generic components originating in three originally separate cell linages. These triomas are stable, immortalized cells which result from the fusion of a human-murine hybridoma with a human lymphoid cell.

As used herein, "tetroma" refers to a a cell line which contains generic components originating in four originally separate cell lineages. These tetromas are stable, immortalized antibody producing cells which result from the fusion of a trioma with a human lymphoid cell which is capable of producing antibody.

As used herein, "autologously" refers to a situation where the same subject is both the source of cell immunoglobulin and the target for cells, or immunoglobulin or therapeutic composition.

As used herein, "heterologously" refers to a situation where one subject is the source of cells or immunoglobulin and another subject is the target for the cell, immunoglobulin or therapeutic composition.

In the practice of any of the methods of the invention or preparation of any of the pharmaceutical compositions a "therapeutically effective amount" is an amount which is capable of binding to an antigen associated with the condition. Accordingly, the effective amount will vary with the subject being treated, as well as the condition to be treated. For the purposes of this invention, the methods of administration are to include, but are not limited to, administration cutaneously, subcutaneously, intravenously, parenterally, orally, topically, or by aerosol.

As used herein, the term "suitable pharmaceutically acceptable carrier" encompasses any of the standard pharmaceutically accepted carriers, such as phosphate buffered saline solution, water, emulsions such as an oil/water emulsion or a triglyceride emulsion, various types of wetting agents, liposomes, tablets, coated tablets, capsules and RBC shadows. An example of an acceptable triglyceride emulsion useful in intravenous and

intraperitoneal administration of the compounds is the triglyceride emulsion commercially known as Intralipid®.

Typically such carriers contain excipients such as starch,
5 milk, sugar, certain types of clay, gelatin, stearic acid,
talc, vegetable fats or oils, gums, glycols, or other known
excipients. Such carriers may also include flavor and
color additives or other ingredients.

10 This invention also provides for pharmaceutical
compositions capable of binding to an antigen associated
with the condition together with suitable diluents,
preservatives, solubilizers, emulsifiers, adjuvants and/or
carriers. Such compositions are liquids or lyophilized or
15 otherwise dried formulations and include diluents of
various buffer content (e.g., Tris-HCl., acetate,
phosphate), pH and ionic strength, additives such as
albumin or gelatin to prevent absorption to surfaces,
detergents (e.g., Tween 20, Tween 80, Pluronic F68, bile
20 acid salts), solubilizing agents (e.g., glycerol,
polyethylene glycerol), anti-oxidants (e.g., ascorbic acid,
sodium metabisulfite), preservatives (e.g., Thimerosal,
benzyl alcohol, parabens), bulking substances or tonicity
modifiers (e.g., lactose, mannitol), covalent attachment of
25 polymers such as polyethylene glycol to the compound,
complexation with metal ions, or incorporation of the
compound into or onto particulate preparations of polymeric
compounds such as polylactic acid, polglycolic acid,
hydrogels, etc, or onto liposomes, micro emulsions,
30 micelles, unilamellar or multi lamellar vesicles,
erythrocyte ghosts, or spheroplasts. Such compositions
will influence the physical state, solubility, stability,
rate of *in vivo* release, and rate of *in vivo* clearance of
the compound or composition.

35 Controlled or sustained release compositions include
formulation in lipophilic depots (e.g., fatty acids, waxes,
oils). Also comprehended by the invention are particulate

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compositions coated with polymers (e.g., poloxamers or poloxamines) and the compound coupled to antibodies directed against tissue-specific receptors, ligands or antigens or coupled to ligands of tissue-specific receptors. Other embodiments of the compositions of the invention incorporate particulate forms protective coatings, protease inhibitors or permeation enhancers for various routes of administration, including parenteral, pulmonary, nasal and oral.

When administered, compounds are often cleared rapidly from the circulation and may therefore elicit relatively short-lived pharmacological activity. Consequently, frequent injections of relatively large doses of bioactive compounds may be required to sustain therapeutic efficacy. Compounds modified by the covalent attachment of water-soluble polymers such as polyethylene glycol, copolymers of polyethylene glycol and polypropylene glycol, carboxymethyl cellulose, dextran, polyvinyl alcohol, polyvinylpyrrolidone or polyproline are known to exhibit substantially longer half-lives in blood following intravenous injection than do the corresponding unmodified compounds (Abuchowski et al., 1981; Newmark et al., 1982; and Katre et al., 1987). Such modifications may also increase the compound's solubility in aqueous solution, eliminate aggregation, enhance the physical and chemical stability of the compound, and greatly reduce the immunogenicity and reactivity of the compound. As a result, the desired *in vivo* biological activity may be achieved by the administration of such polymer-compound adducts less frequently or in lower doses than with the unmodified compound.

Attachment of polyethylene glycol (PEG) to compounds is particularly useful because PEG has very low toxicity in mammals (Carpenter et al., 1971). For example, a PEG adduct of adenosine deaminase was approved in the United States for use in humans for the treatment of severe combined immunodeficiency syndrome. A second advantage

afforded by the conjugation of PEG is that of effectively reducing the immunogenicity and antigenicity of heterologous compounds. For example, a PEG adduct of a human protein might be useful for the treatment of disease in other mammalian species without the risk of triggering a severe immune response. The carrier includes a microencapsulation device so as to reduce or prevent an host immune response against the compound or against cells which may produce the compound. The compound of the present invention may also be delivered microencapsulated in a membrane, such as a liposome.

Polymers such as PEG may be conveniently attached to one or more reactive amino acid residues in a protein such as the alpha-amino group of the amino terminal amino acid, the epsilon amino groups of lysine side chains, the sulfhydryl groups of cysteine side chains, the carboxyl groups of aspartyl and glutamyl side chains, the alpha-carboxyl group of the carboxy-terminal amino acid, tyrosine side chains, or to activated derivatives of glycosyl chains attached to certain asparagine, serine or threonine residues.

Numerous activated forms of PEG suitable for direct reaction with proteins have been described. Useful PEG reagents for reaction with protein amino groups include active esters of carboxylic acid or carbonate derivatives, particularly those in which the leaving groups are N-hydroxysuccinimide, p-nitrophenol, imidazole or 1-hydroxy-2-nitrobenzene-4-sulfonate. PEG derivatives containing maleimido or haloacetyl groups are useful reagents for the modification of protein free sulfhydryl groups. Likewise, PEG reagents containing amino hydrazine or hydrazide groups are useful for reaction with aldehydes generated by periodate oxidation of carbohydrate groups in proteins.

The present invention describes the production of human monoclonal antibodies directed to tumor-associated antigens, tumor cells, infectious agents,

infection-specific antigens, and self antigens using a modified cell fusion partner, trioma cell line and human lymphocytes derived from lymph nodes, spleen, Peyer's patches, or any other lymph tissue or peripheral blood of the human subjects.

Antibodies are selected using cultured cells, purified antigens, primary human cells and tissues and combinatorial libraries relevant to the antibody screening including cells and tissues obtained from autologous donor of lymphoid cells.

The present invention provides a monoclonal antibody which specifically binds and forms a complex with TIP-2 antigen located on the surface of human cancer cells, the TIP-2 antigen being an antigen to which monoclonal antibody 27.B1 specifically binds. According to certain embodiments of the present invention, the monoclonal antibody of the invention is a murine monoclonal antibody, a chimaeric monoclonal antibody, a humanized monoclonal antibody, or a human monoclonal antibody. In an embodiment of the present invention, the monoclonal antibody of the invention is capable of binding to the epitope which is specifically recognized by monoclonal antibody 27.B1 produced by the hybridoma having ATCC Accession No. _____

The present invention provides the monoclonal antibody 27.B1 produced by the hybridoma having ATCC Accession No. _____

The present invention provides a hybridoma cell producing the monoclonal antibody of this invention. In an embodiment of the invention, the hybridoma cell has ATCC Accession No. _____

Hybridoma 27.B1 was deposited on March 28, 2000 with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Va, U.S.A. under the provisions of the

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Budapest Treaty for the International Recognition of the
Deposit of Microorganism for the Purposes of Patent
Procedure. 27.B1 was accorded ATCC Accession Number

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In an embodiment of this invention, a monoclonal antibody
of the invention is labelled with a detectable marker. In
another embodiment of the invention, the detectable marker
is a radioactive isotope, enzyme, dye, biotin, fluorescent
label or chemiluminescent label. In another embodiment of
the invention, the monoclonal antibody is conjugated to a
therapeutic agent. In another embodiment of the invention,
the therapeutic agent is a radioisotope, toxin, toxoid or
chemotherapeutic agent. In another embodiment of the
invention, the monoclonal antibody is conjugated to an
imaging agent. In yet another embodiment of the invention,
the imaging agent is a radioisotope.

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The present invention provides a pharmaceutical composition
comprising the monoclonal antibody of this invention and a
pharmaceutically acceptable carrier.

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The present invention provides a vaccine comprising the
monoclonal antibody of this invention and a
pharmaceutically acceptable carrier.

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The present invention provides a monoclonal antibody which
specifically binds and forms a complex with TIP-2 antigen
located on the surface of human cancer cells, the TIP-2
antigen being an antigen to which monoclonal antibody 27.F7
specifically binds. According to certain embodiments of
the present invention, the monoclonal antibody of the
invention is a murine monoclonal antibody, a chimaeric
monoclonal antibody, a humanized monoclonal antibody, or a
human monoclonal antibody. In an embodiment of the present
invention, the monoclonal antibody of the invention is
capable of binding to the epitope which is specifically

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recognized by monoclonal antibody 27.F7 produced by the hybridoma having ATCC Accession No. _____

5 The present invention provides the monoclonal antibody 27.F7 produced by the hybridoma having ATCC Accession No. _____

10 The present invention provides a hybridoma cell producing the monoclonal antibody of this invention. In an embodiment of the invention, the hybridoma cell has ATCC Accession No. _____

15 Hybridoma 27.F7 was deposited on March 28, 2000 with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Va, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganism for the Purposes of Patent Procedure. 27.F7 was accorded ATCC Accession Number _____

20 In an embodiment of this invention, a monoclonal antibody of the invention is labelled with a detectable marker. In another embodiment of the invention, the detectable marker is a radioactive isotope, enzyme, dye, biotin, fluorescent label or chemiluminescent label. In another embodiment of the invention, the monoclonal antibody is conjugated to a therapeutic agent. In another embodiment of the invention, the therapeutic agent is a radioisotope, toxin, toxoid or chemotherapeutic agent. In another embodiment of the invention, the monoclonal antibody is conjugated to an imaging agent. In yet another embodiment of the invention, the imaging agent is a radioisotope.

30 The present invention provides a pharmaceutical composition comprising the monoclonal antibody of this invention and a pharmaceutically acceptable carrier.

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The present invention provides a vaccine comprising the monoclonal antibody of this invention and a pharmaceutically acceptable carrier.

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The present invention provides a method of detecting TIP-2 antigen bearing cancer cells in a sample comprising: (a) contacting the sample with an antibody directed to an epitope on TIP-2 antigen, or an Fab fragment of an antibody directed to an epitope on TIP-2 antigen, which epitope is recognized by the antibody or the Fab fragment, said antibody or Fab fragment being detectably labeled, under appropriate conditions to produce an antibody/Fab fragment-antigen complex comprising the detectably labeled antibody or Fab fragment bound to any TIP-2 antigen on the surface of cells in the sample; (b) removing any labeled antibody/Fab fragment not bound in the antibody/Fab fragment-antigen complex formed in step (a); and (c) determining presence of the antibody/Fab fragment-antigen complex by detecting the label of the detectably labeled antibody, presence of antibody/Fab fragment-antigen complex indicating TIP-2 antigen-bearing cancer cells in the sample.

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As used herein, "antibody/Fab fragment" means antibody or Fab fragment of the antibodies.

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In the practice of any of the methods of the invention, the unbound antibody or its fragment are usually removed by thorough washing of the sample under testing.

In the practice of any of the methods of the invention, it is more economical to first prepare the fragment and then label it with the label of interest.

In an embodiment of this invention the detectable label is selected from the group consisting of radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

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In an embodiment of this invention the TIP-2 antigen-bearing cancer cells are human cancer cells.

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In an embodiment of this invention the cancer cells are selected from a group consisting of melanoma cells, basal cell carcinoma cells, squamous cell carcinoma cells, neuroblastoma cells, glioblastoma multiforme cells, myeloid leukemic cells, breast carcinoma cells, colon carcinoma cells, endometrial carcinoma cells, lung carcinoma cells, ovarian carcinoma cells, prostate carcinoma cells, cervical carcinoma cells, osteosarcoma cells, testicular carcinoma cells and lymphoma cells.

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In an embodiment of this invention the antibody is a monoclonal antibody.

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In an embodiment of this invention the antibody is a human monoclonal antibody or a murine monoclonal antibody.

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In an embodiment of this invention the sample is selected from the group consisting of serum, plasma, saliva, tears, mucosal discharge, urine, peritoneal fluid, cerebrospinal fluid, lymphatic fluid, bone marrow, breast biopsy, tissue, lymph nodes, prostate tissue, tissues from breast and prostate metastases, culture media, and other tumors where TIP-2 can be an associated antigen.

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In an embodiment of this invention TIP-2 is concentrated from the sample by alcohol precipitation prior to step (a).

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In an embodiment of this invention the sample is culture media.

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The present invention provides a method of detecting TIP-2 antigen bearing cancer cells in a sample comprising: (a) contacting the sample with an antibody directed to an epitope on TIP-2 antigen, or an Fab fragment of an antibody directed to an epitope on TIP-2 antigen, which epitope is recognized by the antibody or the Fab fragment under appropriate conditions to produce an antibody/Fab fragment-antigen complex comprising the antibody or Fab fragment bound to any TIP-2 antigen on the surface of cells in the sample; (b) removing any antibody/Fab fragment not bound in the antibody/Fab fragment-antigen complex formed in step (a); (c) contacting the antibody/Fab fragment-antigen complex of step (b) with a second antibody which specifically binds to the antibody/Fab fragment-antigen complex, said second antibody being detectably labeled, under appropriate conditions to permit the second labeled antibody to bind to the antibody/Fab fragment-antigen complex; (d) removing any second labeled antibody not bound to the antibody/Fab fragment-antigen complex product in (c); and (e) determining presence of the antibody/Fab fragment-antigen complex bound to the second labeled antibody by detecting the label of second antibody, presence of antibody/Fab fragment-antigen complex indicating TIP-2 antigen-bearing human cancer cells in the sample.

In an embodiment of this invention the detectable label is radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

In an embodiment of this invention the TIP-2 antigen-bearing cancer cells are human cancer cells.

In an embodiment of this invention the cancer cells are selected from a group consisting of melanoma cells, basal cell carcinoma cells, squamous cell carcinoma cells, neuroblastoma cells, glioblastoma multiforme cells, myeloid leukemic cells, breast carcinoma cells, colon carcinoma

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In an embodiment of this invention the antibody is a monoclonal antibody.

In an embodiment of this invention the antibody is a human monoclonal antibody or a murine monoclonal antibody.

In an embodiment of this invention the sample is selected from the group consisting of serum, plasma, saliva, tears, mucosal discharge, urine, peritoneal fluid, cerebrospinal fluid, lymphatic fluid, bone marrow, breast biopsy, tissue, lymph nodes, prostate tissue, tissues from breast and prostate metastases, culture media, and other tumors where TIP-2 can be an associated antigen.

In an embodiment of this invention TIP-2 is concentrated from the sample by alcohol precipitation prior to step (a).

The present invention provides a method of detecting TIP-2 antigen on the surface of cancer cells in a sample comprising: (a) contacting the sample with an antibody directed to an epitope on TIP-2 antigen or an Fab fragment thereof, which epitope is recognized by monoclonal antibody 27.F7 produced by the hybridoma designated _____, said antibody or Fab fragment thereof being detectably labeled, under appropriate conditions to produce an antibody 27.F7/Fab fragment-TIP-2 antigen complex comprising the detectably labeled antibody bound to any TIP-2 antigen on the surface of cells in the sample; b) removing any labeled antibody/Fab fragment not bound in the antibody 27.F7/Fab fragment-TIP-2 antigen complex formed in step (a); and (c) determining presence of the antibody 27.F7/Fab fragment-TIP-2 antigen complex by detecting the label of the detectably labeled antibody, presence of antibody 27.F7/Fab

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fragment-TIP-2 antigen complex indicating TIP-2 antigen-bearing human cancer cells in the sample.

5 In an embodiment of this invention the detectable label is selected from the group consisting of radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

10 In an embodiment of this invention the TIP-2 antigen-bearing cancer cells are human cancer cells.

15 In an embodiment of this invention the cancer cells are selected from a group consisting of melanoma cells, basal cell carcinoma cells, squamous cell carcinoma cells, neuroblastoma cells, glioblastoma multiforme cells, myeloid leukemic cells, breast carcinoma cells, colon carcinoma cells, endometrial carcinoma cells, lung carcinoma cells, ovarian carcinoma cells, prostate carcinoma cells, cervical carcinoma cells, osteosarcoma cells, testicular carcinoma cells and lymphoma cells.

20 In an embodiment of this invention the antibody is a monoclonal antibody.

25 In an embodiment of this invention the antibody is a human monoclonal antibody or a murine monoclonal antibody.

30 In an embodiment of this invention the sample is selected from the group consisting of serum, plasma, saliva, tears, mucosal discharge, urine, peritoneal fluid, cerebrospinal fluid, lymphatic fluid, bone marrow, breast biopsy, tissue, lymph nodes, prostate tissue, tissues from breast and prostate metastases, culture media, and other tumors where TIP-2 can be an associated antigen.

35 In an embodiment of this invention TIP-2 is concentrated from the sample by alcohol precipitation prior to step (a).

The present invention provides a method of detecting TIP-2 antigen on the surface of cancer cells in a sample comprising: (a) contacting the sample with an antibody directed to an epitope on TIP-2 antigen which epitope is recognized by monoclonal antibody 27.F7 produced by the hybridoma designated _____ or Fab fragment thereof, under appropriate conditions to produce an antibody 27.F7/Fab fragment-TIP-2 antigen complex comprising the antibody bound to any TIP-2 antigen on the surface of cells in the sample;

(b) removing any antibody or Fab fragment thereof not bound in the antibody 27.F7/Fab fragment-TIP-2 antigen complex formed in step (a); (c) contacting the antibody 27.F7/Fab fragment-TIP-2 antigen complex of step (b) with a second antibody which specifically binds to the antibody 27.F7/Fab fragment-TIP-2 antigen complex, said second antibody being detectably labeled, under appropriate conditions to permit the second labeled antibody to bind to the antibody 27.F7/Fab fragment-TIP-2 antigen complex; (d) removing any second labeled antibody not bound to the antibody 27.F7/Fab fragment-TIP-2 antigen complex product in (c); and (e) determining presence of the antibody 27.F7/Fab fragment-TIP-2 antigen complex bound to the second labeled antibody by detecting the label of second antibody, presence of antibody 27.F7/Fab fragment-TIP-2 antigen complex indicating TIP-2 antigen-bearing human cancer cells in the sample.

In an embodiment of this invention the detectable label is radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

In an embodiment of this invention the TIP-2 antigen-bearing cancer cells are human cancer cells.

In an embodiment of this invention the cancer cells are selected from a group consisting of melanoma cells, basal cell carcinoma cells, squamous cell carcinoma cells,

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In an embodiment of this invention TIP-2 is concentrated from the sample by alcohol precipitation prior to step (a).

5 The present invention provides a method of detecting TIP-2 antigen on the surface of cancer cells in a sample comprising: (a) contacting the sample with an antibody directed to an epitope on TIP-2 antigen or an Fab fragment thereof, which epitope is recognized by monoclonal antibody 27.B1 produced by the hybridoma designated _____, or Fab
10 fragment thereof under appropriate conditions to produce an antibody 27.B1/Fab fragment-TIP-2 antigen complex comprising the antibody bound to any TIP-2 antigen on the surface of cells in the sample; (b) removing any antibody/Fab fragment thereof not bound in the antibody 27.B1/Fab fragment-TIP-2 antigen complex formed in step
15 (a); (c) contacting the antibody 27.B1/Fab fragment-TIP-2 antigen complex of step (b) with a second antibody which specifically binds to the antibody 27.B1/Fab fragment-TIP-2 antigen complex, said second antibody being detectably labeled, under appropriate conditions to permit the second
20 labeled antibody to bind to the antibody 27.B1/Fab fragment-TIP-2 antigen complex; (d) removing any second labeled antibody not bound to the antibody 27.B1/Fab fragment-TIP-2 antigen complex product in (c); and (e)
25 determining presence of the antibody 27.B1/Fab fragment-TIP-2 antigen complex bound to the second labeled antibody by detecting the label of second antibody, presence of antibody 27.B1/Fab fragment-TIP-2 antigen complex indicating TIP-2 antigen-bearing human cancer cells in the
30 sample.

In an embodiment of this invention the detectable label is radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

35 In an embodiment of this invention the TIP-2 antigen-bearing cancer cells are human cancer cells.

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In an embodiment of this invention the cancer cells are selected from a group consisting of human melanoma cells, basal cell carcinoma cells, squamous cell carcinoma cells, neuroblastoma cells, glioblastoma multiforme cells, myeloid
5 leukemic cells, breast carcinoma cells, colon carcinoma cells, endometrial carcinoma cells, lung carcinoma cells, ovarian carcinoma cells, prostate carcinoma cells, cervical carcinoma cells, osteosarcoma cells, testicular carcinoma cells and lymphoma cells.

10 In an embodiment of this invention the antibody is a monoclonal antibody.

15 In an embodiment of this invention the antibody is a human monoclonal antibody or a murine monoclonal antibody.

20 In an embodiment of this invention the sample is selected from the group consisting of serum, plasma, saliva, tears, mucosal discharge, urine, peritoneal fluid, cerebrospinal fluid, lymphatic fluid, bone marrow, breast biopsy, tissue, lymph nodes, prostate tissue, tissues from breast and prostate metastases, culture media, and other tumors where TIP-2 can be an associated antigen.

25 In an embodiment of this invention TIP-2 is concentrated from the sample by alcohol precipitation prior to step (a).

30 *Emb 24*
~~The present invention provides a method for diagnosing cancer in a subject by detecting TIP-2 antigen-bearing cancer cells which comprises: (a) obtaining a sample of the subject's peripheral blood; (b) contacting the sample with an antibody directed to an epitope on TIP-2 antigen or an Fab fragment thereof, which epitope is recognized by monoclonal antibody 27.F7 produced by the hybridoma designated _____ or an Fab fragment thereof, said
35 antibody being detectably labeled, under appropriate conditions to produce an antibody 27.F7/Fab fragment-TIP-2 antigen complex comprising the detectably labeled antibody~~

bound to any TIP-2 antigen on the surface of cells in the sample; (c) removing any labeled antibody/Fab fragment not bound in the antibody 27.F7/Fab fragment-TIP-2 antigen complex formed in step (b); and (d) determining presence of the antibody 27.F7/Fab fragment-TIP-2 antigen complex by detecting the label of the detectably labeled antibody, presence of antibody 27.F7/Fab fragment-TIP-2 antigen complex indicating diagnosis of cancer in the subject.

10 In an embodiment of this invention the detectable label is radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

15 In an embodiment of this invention the subject is human.

20 In an embodiment of this invention the cancer is human melanoma, basal cell carcinoma, squamous cell carcinoma, neuroblastoma, glioblastoma multiforme, myeloid leukemia, breast carcinoma, colon carcinoma, endometrial carcinoma, lung carcinoma, ovarian carcinoma, prostate carcinoma, cervical carcinoma, osteosarcoma, testicular carcinoma and lymphoma.

25 In an embodiment of this invention the antibody is a monoclonal antibody.

In an embodiment of this invention the antibody is a human monoclonal antibody or a murine monoclonal antibody.

30 In an embodiment of this invention the sample is selected from the group consisting of serum, plasma, saliva, tears, mucosal discharge, urine, peritoneal fluid, cerebrospinal fluid, lymphatic fluid, bone marrow, breast biopsy, tissue, lymph nodes, prostate tissue, tissues from breast and prostate metastases, culture media, and other tumors where TIP-2 can be an associated antigen.

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In an embodiment of this invention TIP-2 is concentrated from the sample by alcohol precipitation prior to step (a).

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5 The present invention provides a method for diagnosing cancer in a subject by detecting TIP-2 antigen-bearing cancer cells which comprises: (a) obtaining a sample of the subject's peripheral blood; (b) contacting the sample with an antibody directed to an epitope on TIP-2 antigen or Fab fragment thereof, which epitope is recognized by monoclonal antibody 27.F7 produced by the hybridoma designated _____ or Fab fragment thereof, under appropriate conditions to produce an antibody 27.F7/Fab fragment-TIP-2 antigen complex comprising the antibody bound to any TIP-2 antigen on the surface of cells in the sample; (c) removing any antibody/Fab fragment not bound in the antibody 27.F7/Fab fragment-TIP-2 antigen complex formed in step (b); (d) contacting the antibody 27.F7/Fab fragment-TIP-2 antigen complex of step (c) with a second antibody which specifically binds to the antibody 27.F7/Fab fragment-TIP-2 antigen complex, said second antibody being detectably labeled, under appropriate conditions to permit the second labeled antibody to bind to the antibody 27.F7/Fab fragment-TIP-2 antigen complex; (e) removing any second labeled antibody not bound to the antibody 27.F7/Fab fragment-TIP-2 antigen complex product in (d); and (f) determining presence of the antibody 27.F7/Fab fragment-TIP-2 antigen complex bound to the second labeled antibody by detecting the label of second antibody, presence of antibody 27.F7/Fab fragment-TIP-2 antigen complex indicating diagnosis of cancer in the subject.

In an embodiment of this invention the detectable label is radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

In an embodiment of this invention the subject is human.

In an embodiment of this invention the cancer is human melanoma, basal cell carcinoma, squamous cell carcinoma, neuroblastoma, glioblastoma multiforme, myeloid leukemia, breast carcinoma, colon carcinoma, endometrial carcinoma, lung carcinoma, ovarian carcinoma, prostate carcinoma, cervical carcinoma, osteosarcoma, testicular carcinoma and lymphoma.

In an embodiment of this invention the antibody is a monoclonal antibody.

In an embodiment of this invention the antibody is a human monoclonal antibody or a murine monoclonal antibody.

In an embodiment of this invention the sample is selected from the group consisting of serum, plasma, saliva, tears, mucosal discharge, urine, peritoneal fluid, cerebrospinal fluid, lymphatic fluid, bone marrow, breast biopsy, tissue, lymph nodes, prostate tissue, tissues from breast and prostate metastases, culture media, and other tumors where TIP-2 can be an associated antigen.

In an embodiment of this invention TIP-2 is concentrated from the sample by alcohol precipitation prior to step (a).

The present invention provides a method for diagnosing cancer in a subject by detecting TIP-2 antigen-bearing cancer cells which comprises: (a) obtaining a sample of the subject's peripheral blood; (b) contacting the sample with an antibody directed to an epitope on TIP-2 antigen or an Fab fragment thereof, which epitope is recognized by monoclonal antibody 27.B1 produced by the hybridoma designated _____, said antibody being detectably labeled, under appropriate conditions to produce an antibody 27.B1/Fab fragment-TIP-2 antigen complex comprising the detectably labeled antibody bound to any TIP-2 antigen on the surface of cells in the sample; (c) removing any labeled antibody/Fab fragment not bound in the antibody 27.B1/Fab

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(16)

5 fragment-TIP-2 antigen complex formed in step (b); and (d) determining presence of the antibody 27.B1/Fab fragment-TIP-2 antigen complex by detecting the label of the detectably labeled antibody, presence of antibody 27.B1/Fab fragment-TIP-2 antigen complex indicating diagnosis of cancer in the subject.

10 In an embodiment of this invention the detectable label is radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

In an embodiment of this invention the subject is human.

15 In an embodiment of this invention the cancer is human melanoma, basal cell carcinoma, squamous cell carcinoma, neuroblastoma, glioblastoma multiforme, myeloid leukemia, breast carcinoma, colon carcinoma, endometrial carcinoma, lung carcinoma, ovarian carcinoma, prostate carcinoma, cervical carcinoma, osteosarcoma, testicular carcinoma and
20 lymphoma.

In an embodiment of this invention the antibody is a monoclonal antibody.

25 In an embodiment of this invention the antibody is a human monoclonal antibody or a murine monoclonal antibody.

30 In an embodiment of this invention the sample is selected from the group consisting of serum, plasma, saliva, tears, mucosal discharge, urine, peritoneal fluid, cerebrospinal fluid, lymphatic fluid, bone marrow, breast biopsy, tissue, lymph nodes, prostate tissue, tissues from breast and prostate metastases, culture media, and other tumors where TIP-2 can be an associated antigen.

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5 The present invention provides a method for diagnosing
cancer in a subject by detecting TIP-2 antigen-bearing
cancer cells which comprises: (a) obtaining a sample of the
subject's peripheral blood; (b) contacting the sample with
an antibody directed to an epitope on TIP-2 antigen or Fab
10 fragment thereof, which epitope is recognized by monoclonal
antibody 27.B1/Fab fragment produced by the hybridoma
designated _____ or Fab fragment thereof, under
appropriate conditions to produce an antibody 27.B1/Fab
fragment-TIP-2 antigen complex comprising the antibody
bound to any TIP-2 antigen on the surface of cells in the
15 sample; (c) removing any antibody/Fab fragment not bound in
the antibody 27.B1/Fab fragment-TIP-2 antigen complex
formed in step (b); (d) contacting the antibody 27.B1/Fab
fragment-TIP-2 antigen complex of step (c) with a second
antibody which specifically binds to the antibody 27.B1/Fab
20 fragment-TIP-2 antigen complex, said second antibody being
detectably labeled, under appropriate conditions to permit
the second labeled antibody to bind to the antibody
27.B1/Fab fragment-TIP-2 antigen complex; (e) removing any
second labeled antibody not bound to the antibody 27.B1/Fab
25 fragment-TIP-2 antigen complex product in (d); and (f)
determining presence of the antibody 27.B1/Fab fragment-
TIP-2 antigen complex bound to the second labeled antibody
by detecting the label of second antibody, presence of
antibody 27.B1/Fab fragment-TIP-2 antigen complex
30 indicating diagnosis of cancer in the subject.

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In an embodiment of this invention the subject is human.

In an embodiment of this invention the cancer is human melanoma, basal cell carcinoma, squamous cell carcinoma, neuroblastoma, glioblastoma multiforme, myeloid leukemia, breast carcinoma, colon carcinoma, endometrial carcinoma, lung carcinoma, ovarian carcinoma, prostate carcinoma, cervical carcinoma, osteosarcoma, testicular carcinoma and lymphoma.

In an embodiment of this invention the antibody is a monoclonal antibody.

In an embodiment of this invention the antibody is a human monoclonal antibody or a murine monoclonal antibody.

In an embodiment of this invention the sample is selected from the group consisting of serum, plasma, saliva, tears, mucosal discharge, urine, peritoneal fluid, cerebrospinal fluid, lymphatic fluid, bone marrow, breast biopsy, tissue, lymph nodes, prostate tissue, tissues from breast and prostate metastases, culture media, and other tumors where TIP-2 can be an associated antigen.

In an embodiment of this invention TIP-2 is concentrated from the sample by alcohol precipitation prior to step (a).

The present invention provides an in vivo method for diagnosing cancer in a subject by detecting TIP-2 antigen-bearing cancer cells which comprises: (a) administering to the subject an antibody directed to an epitope on TIP-2 antigen or Fab fragment thereof, which epitope is recognized by monoclonal antibody 27.F7 produced by the hybridoma designated _____, said antibody being detectably labeled, under appropriate conditions to bind the antibody to TIP-2 antigen on the surface of any cells in the subject; and (b) determining presence of the detectably labeled antibody 27.F7 bound to the surface of cells in the subject, presence of detectably labeled

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antibody 27.F7 bound to cells indicating diagnosis of cancer in the subject.

5 In an embodiment of this invention the detectable label is radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

In an embodiment of this invention the subject is human.

10 In an embodiment of this invention the cancer is human melanoma, basal cell carcinoma, squamous cell carcinoma, neuroblastoma, glioblastoma multiforme, myeloid leukemia, breast carcinoma, colon carcinoma, endometrial carcinoma, lung carcinoma, ovarian carcinoma, prostate carcinoma,
15 cervical carcinoma, osteosarcoma, testicular carcinoma and lymphoma.

In an embodiment of this invention the antibody is a monoclonal antibody.

20 In an embodiment of this invention the antibody is a human monoclonal antibody or a murine monoclonal antibody.

25 In an embodiment of this invention in step (b) presence of the antibody 27.F7 or Fab fragment thereof bound to the surface of cells in the subject is detected wherein means for detecting the detectable label is an imaging device.

30 In an embodiment of this invention the imaging device is magnetic resonance imaging device.

In an embodiment of this invention the imaging device is X-ray immunoscintigraphy-imaging device.

35 The present invention provides an in vivo method for diagnosing cancer in a subject by detecting TIP-2 antigen-bearing cancer cells which comprises: (a) administering to the subject an antibody directed to an epitope on TIP-2

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antigen or Fab fragment thereof, which epitope is recognized by monoclonal antibody 27.B1 produced by the hybridoma designated _____, said antibody/Fab fragment being detectably labeled, under appropriate conditions to bind the antibody to TIP-2 antigen on the surface of any cells in the subject; and (b) determining presence of the detectably labeled antibody/Fab fragment 27.B1 bound to the surface of cells in the subject, presence of detectably labeled antibody 27.F7/Fab fragment bound to cells indicating diagnosis of cancer in the subject.

In an embodiment of this invention the detectable label is radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

In an embodiment of this invention the subject is human.

In an embodiment of this invention the cancer is human melanoma, basal cell carcinoma, squamous cell carcinoma, neuroblastoma, glioblastoma multiforme, myeloid leukemia, breast carcinoma, colon carcinoma, endometrial carcinoma, lung carcinoma, ovarian carcinoma, prostate carcinoma, cervical carcinoma, osteosarcoma, testicular carcinoma and lymphoma.

In an embodiment of this invention the antibody is a monoclonal antibody.

In an embodiment of this invention the antibody is a human monoclonal antibody or a murine monoclonal antibody.

In an embodiment of this invention the sample is selected from the group consisting of serum, plasma, saliva, tears, mucosal discharge, urine, peritoneal fluid, cerebrospinal fluid, lymphatic fluid, bone marrow, breast biopsy, tissue, lymph nodes, prostate tissue, tissues from breast and prostate metastases, culture media, and other tumors where TIP-2 can be an associated antigen.

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In an embodiment of this invention in step (b) presence of the antibody 27.B1 or fragment thereof bound to the surface of cells in the subject is detected by means for detecting the detectable label is an imaging device.

In an embodiment of this invention the imaging device is magnetic resonance imaging device.

In an embodiment of this invention the imaging device is X-ray immunoscintigraphy-imaging device.

The present invention provides a method for delivering exogenous material to TIP-2 antigen-bearing cancer cells of a human subject comprising administering to the subject a liposome carrying a conjugate of the exogenous material, wherein antibody 27.B1 or an Fab fragment of 27.B1 is coupled to the outer surface of the liposome to target delivery to the cancer cells.

In an embodiment of this invention the exogenous material is selected from the group consisting of anti-cancer drugs, radioisotopes, toxins, antibiotics, prodrugs, enzymes, and chemotherapeutic compounds.

In an embodiment of this invention the TIP-2 antigen-bearing cancer cells are human melanoma cells, basal cell carcinoma cells, squamous cell carcinoma cells, neuroblastoma cells, glioblastoma multiforme cells, myeloid leukemic cells, breast carcinoma cells, colon carcinoma cells, endometrial carcinoma cells, lung carcinoma cells, ovarian carcinoma cells, prostate carcinoma cells, cervical carcinoma cells, osteosarcoma cells, testicular carcinoma cells and lymphoma cells.

The present invention provides a method for delivering exogenous material to TIP-2 antigen-bearing cancer cells of a human subject comprising administering to the subject a

liposome carrying a conjugate of the exogenous material, wherein an antibody 27.F7 or an Fab fragment of 27.F7 is coupled to the outer surface of the liposome to target delivery to the cancer cells.

5

In an embodiment of this invention the exogenous material is selected from the group consisting of anti-cancer drugs, radioisotopes, toxins, antibiotics, prodrugs, enzymes, and chemotherapeutic compounds.

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In an embodiment of this invention the TIP-2 antigen-bearing cancer cells are human melanoma cells, basal cell carcinoma cells, squamous cell carcinoma cells, neuroblastoma cells, glioblastoma multiforme cells, myeloid leukemic cells, breast carcinoma cells, colon carcinoma cells, endometrial carcinoma cells, lung carcinoma cells, ovarian carcinoma cells, prostate carcinoma cells, cervical carcinoma cells, osteosarcoma cells, testicular carcinoma cells and lymphoma cells.

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The present invention provides a method for treating cancer in a human subject by evoking a specific immune response which comprises administering to the subject a whole TIP-2 antigen protein or a peptide fragment of TIP-2 to the subject.

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In the above-described method, the whole TIP-2 or TIP-2 derived peptides can be either (1) injected directly or (2) coupled to a carrier protein or (3) in a mixture with adjuvant or (4) otherwise modified (such as by coupling to tetanus toxoid) to boost the immune response directed to all TIP-2 bearing cells.

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In an embodiment of this invention the specific immune response is complement-dependent cytolysis of TIP-2 antigen-bearing cancer cells.

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In an embodiment of this invention the specific immune response is activation of natural killer cells towards TIP-2 antigen-bearing cancer cells.

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In an embodiment of this invention the peptide fragment of TIP-2 antigen comprises the amino acid sequence Lys Leu Leu Gly Gly Gln Ile Gly Leu (SEQ. ID NO.).

10 In an embodiment of this invention the peptide fragment of TIP-2 antigen comprises the amino acid sequence Ser Leu Leu Gly Cys Arg His Tyr Glu Val (SEQ. ID No.).

15 The present invention provides a method for treating cancer in a human subject by inducing apoptosis of cancer cells which comprises administering to the subject a whole TIP-2 antigen protein or a peptide fragment of TIP-2 to the subject.

20 The present invention provides a method for treating cancer in a human subject by evoking a specific immune response which comprises: (a) removing dendritic cells from said subject; (b) contacting the dendritic cells of step (a) with a whole TIP-2 antigen protein or a peptide fragment of TIP-2; and (c) reintroducing the dendritic cells of step
25 (b) into said subject.

In the above-described method, the dendritic cells will present the antigen to the autologous immune system and thereby induce antibodies in the subject.

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In an embodiment of this invention the peptide fragment of TIP-2 antigen comprises the amino acid sequence Lys Leu Leu Gly Gly Gln Ile Gly Leu (SEQ. ID NO.).

35 In an embodiment of this invention the peptide fragment of TIP-2 antigen comprises the amino acid sequence Ser Leu Leu Gly Cys Arg His Tyr Glu Val (SEQ. ID No.).

In an embodiment of this invention the specific immune response is complement-dependent cytolysis of TIP-2 antigen-bearing cancer cells.

5 In an embodiment of this invention the specific immune response is activation of natural killer cells towards TIP-2 antigen-bearing cancer cells.

10 In an embodiment of this invention the specific immune response is the production of antibodies in the subject against the whole TIP-2 antigen protein or the peptide fragment of TIP-2.

15 In the above-described method, antibodies injected into the patient in order to evoke immune response to cancer can be either fully human, humanized, or fragments thereof, either directly or indirectly coupled to a toxin, a drug or a prodrug, an enzyme, a radionuclide, or to liposomes carrying the payload of a drug, toxin, prodrug, enzyme or radionuclide. Such antibodies can evoke the immune response by activating effector cells (natural killer cells and macrophages), causing ADCC; can activate complement, causing CDC, or can act directly through apoptosis. Such antibodies can also induce the cascade of anti-idiotypic antibodies, where Ab2 (mimetics of the antigen, in this case TIP-2) will cause even stronger anti-TIP-2 immune response by inducing Ab3 (mimetics of original anti-TIP-2 Ab1).

30 The present invention provides a method for treating cancer in a human subject by inducing apoptosis of cancer cells which comprises administering a whole TIP-2 antigen protein or a peptide fragment of TIP-2 to the subject.

35 The present invention provides a method for treating cancer in a human subject by passive immunization which comprises administering an antibody directed to an epitope on TIP-2 antigen or a peptide fragment thereof.

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In an embodiment of this invention the antibody induces apoptosis of TIP-2 antigen bearing cells.

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The present invention provides an isolated peptide having the amino acid sequence Lys Leu Leu Gly Gly Gln Ile Gly Leu (SEQ. ID No.).

The present invention provides an isolated peptide having the amino acid sequence Ser Leu Leu Gly Cys Arg His Tyr Glu Val (SEQ. ID No.).

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15 The present invention provides a method for immunohistochemical screening of a tissue section from a tumor sample for the presence of TIP-2 antigen bearing cancer cells which comprises: (a) contacting the tissue section from the tumor sample with an antibody directed to an epitope on TIP-2 antigen or Fab fragment thereof, which epitope is recognized by monoclonal antibody 27.F7 produced by the hybridoma designated _____, said antibody/Fab fragment being detectably labeled, under appropriate conditions to produce an antibody 27.F7/Fab fragment-TIP-2 antigen complex comprising the detectably labeled antibody bound to any TIP-2 antigen on the surface of cells in the tissue section; (a) removing any labeled antibody/Fab fragment not bound in the antibody 27.F7/Fab fragment-TIP-2 antigen complex formed in step (a); and (b) determining presence of the antibody 27.F7/Fab fragment-TIP-2 antigen complex by detecting the label of the detectably labeled antibody, presence of antibody 27.F7/Fab fragment-TIP-2 antigen complex indicating TIP-2 antigen-bearing human cancer cells in the sample.

In an embodiment of this invention the tissue section is preserved freshly frozen tissue or formalin-fixed tissue.

35 In an embodiment of this invention the detectable label is radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

In an embodiment of this invention the TIP-2 antigen-bearing cancer cells are human cancer cells.

5 In an embodiment of this invention the cancer cells are selected from a group consisting of melanoma cells, basal cell carcinoma cells, squamous cell carcinoma cells, neuroblastoma cells, glioblastoma multiforme cells, myeloid leukemic cells, breast carcinoma cells, colon carcinoma cells, endometrial carcinoma cells, lung carcinoma cells, ovarian carcinoma cells, prostate carcinoma cells, cervical carcinoma cells, osteosarcoma cells, testicular carcinoma cells and lymphoma cells. the antibody is a monoclonal antibody.

15 In an embodiment of this invention the antibody is a human monoclonal antibody.

20 The present invention provides a kit for detecting the presence of TIP-2 antigen-bearing cancer cells in a sample comprising: (a) solid support having a plurality of covalently linked probes which may be the same or different, each probe of which comprises a monoclonal antibody directed to an epitope on TIP-2 antigen or Fab fragment thereof; and (b) a means for determining the presence of monoclonal antibody/Fab fragment-TIP-2 antigen complex.

30 In an embodiment of this invention the means for determining the presence of the monoclonal antibody/Fab fragment-TIP-2 antigen complex is a detectably labeled second antibody which specifically binds to the monoclonal antibody directed to the epitope on TIP-2 antigen.

35 In an embodiment of this invention the monoclonal antibody directed to the epitope on TIP-2 antigen is human monoclonal antibody 27.F7 directed to an epitope on TIP-2

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prostate metastases, culture media, and other tumors where TIP-2 can be an associated antigen.

In an embodiment of this invention the sample is culture media.

In an embodiment of this invention the sample is a tumor sample.

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The present invention provides a method for detecting the presence of TIP-2 antigen in biological fluid comprising: (a) contacting a sample of the biological fluid with a antibody directed to an epitope on TIP-2 antigen or Fab fragment thereof, which epitope is recognized by monoclonal antibody 27.F7 produced by the hybridoma designated _____, said antibody being detectably labeled, under appropriate conditions to produce an antibody 27.F7/Fab fragment-TIP-2 antigen complex comprising the detectably labeled antibody bound to any TIP-2 antigen on the surface of cells in the sample; (c) removing any labeled antibody not bound in the antibody 27.F7/Fab fragment-TIP-2 antigen complex formed in step (a); and (d) determining presence of the antibody 27.F7/Fab fragment-TIP-2 antigen complex by detecting the label of the detectably labeled antibody, presence of antibody 27.F7/Fab fragment-TIP-2 antigen complex indicating TIP-2 antigen-bearing human cancer cells in the biological fluid.

In an embodiment of this invention the detectable label is radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

In an embodiment of this invention the TIP-2 antigen-bearing cancer cells are human cancer cells.

In an embodiment of this invention the cancer cells are selected from a group consisting of melanoma cells, basal cell carcinoma cells, squamous cell carcinoma cells,

neuroblastoma cells, glioblastoma multiforme cells, myeloid
leukemic cells, breast carcinoma cells, colon carcinoma
cells, endometrial carcinoma cells, lung carcinoma cells,
ovarian carcinoma cells, prostate carcinoma cells, cervical
5 carcinoma cells, osteosarcoma cells, testicular carcinoma
cells and lymphoma cells.

10 In an embodiment of this invention the biological fluid is
selected from the group consisting of serum, plasma,
saliva, tears, mucosal discharge, urine, peritoneal fluid,
cerebrospinal fluid, and lymphatic fluid.

15 In an embodiment of this invention TIP-2 is concentrated
from the sample by alcohol precipitation prior to step (a).

In an embodiment of this invention the biological fluid is
culture media.

20 In an embodiment of this invention the monoclonal antibody
directed to the epitope on TIP-2 antigen is human
monoclonal antibody 27.F7 directed to an epitope on TIP-2
antigen, which epitope is recognized by monoclonal antibody
27.F7 produced by the hybridoma designated _____.

25 In an embodiment of this invention the monoclonal antibody
directed to the epitope on TIP-2 antigen is human
monoclonal antibody 27.B1 directed to an epitope on TIP-2
antigen, which epitope is recognized by monoclonal antibody
27.B1 produced by the hybridoma designated _____.

30 In an embodiment of this invention the monoclonal antibody
directed to the epitope of TIP-2 antigen is a murine
monoclonal antibody directed to an epitope on TIP-2
antigen.

35 In an embodiment of this invention the TIP-2 antigen is
present on TIP-2 antigen-bearing cancer cells in the
biological fluid.

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5 The present invention provides a method for immunohistochemical screening of tissue sections from a tumor sample for the presence of TIP-2 antigen-bearing cancer cells which comprises: (a) contacting the tissue section from the tumor sample with a detectably labeled antibody directed to an epitope on TIP-2 antigen or Fab fragment thereof, which epitope is recognized by monoclonal antibody 27.B1 produced by the hybridoma designated _____, said antibody being detectably labeled, under appropriate conditions to bind the antibody to TIP-2 antigen on the surface of any cells in the sample; and (b) removing any labeled antibody not bound to the cells in the sample; (c) determining presence of

15 antibody 27.B1 bound to the cells in the sample, presence of antibody 27.B1 bound to cells indicating TIP-2 antigen-bearing cancer cells in the tumor sample.

20 In an embodiment of this invention tissue section is preserved freshly frozen tissue or formalin-fixed tissue.

In an embodiment of this invention the detectable label is radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

25 In an embodiment of this invention the TIP-2 antigen-bearing cancer cells are human cancer cells.

30 In an embodiment of this invention the cancer cells are selected from a group consisting of melanoma cells, basal cell carcinoma cells, squamous cell carcinoma cells, neuroblastoma cells, glioblastoma multiforme cells, myeloid leukemic cells, breast carcinoma cells, colon carcinoma cells, endometrial carcinoma cells, lung carcinoma cells,

35 ovarian carcinoma cells, prostate carcinoma cells, cervical carcinoma cells, osteosarcoma cells, testicular carcinoma cells and lymphoma cells.

In an embodiment of this invention the antibody is a monoclonal antibody.

In an embodiment of this invention the monoclonal antibody
5 is a human monoclonal antibody or a murine monoclonal antibody.

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The present invention provides a method for monitoring progression of cancer, wherein cancer cells are TIP-2 antigen-bearing cancer cells, in a subject comprising: (a) administering to a subject diagnosed with cancer an antibody directed to an epitope on TIP-2 antigen or Fab fragment thereof, which epitope is recognized by monoclonal antibody 27.F7 produced by the hybridoma designated _____, said antibody being detectably labeled, under appropriate conditions to bind the antibody to TIP-2 antigen on the surface of any cells in the subject; (b) determining presence of detectably labeled antibody 27.F7/Fab fragment bound to the surface of cells in the subject according to the according to the method of claim 23; (c) comparing the presence of detectably labeled antibody/Fab fragment 27.F7 bound to cells in step (b) with the presence of detectably labeled antibody 27.F7 bound to cells at (i) diagnosis time or (ii) after treatment, wherein a greater presence of detectably labeled antibody 27.F7/Fab fragment bound to cells in step (b) than at (i) diagnosis time or (ii) after treatment, indicates progression of the cancer in the subject and a lesser presence of detectably labeled antibody 27.F7/Fab fragment bound to cells in step (b) than at (i) diagnosis time or (ii) after treatment indicates regression of the cancer in the subject.

35 In an embodiment of this invention the detectable label is radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

In an embodiment of this invention the TIP-2 antigen-bearing cancer cells are human cancer cells.

5 In an embodiment of this invention the cancer cells are selected from a group consisting of melanoma cells, basal cell carcinoma cells, squamous cell carcinoma cells, neuroblastoma cells, glioblastoma multiforme cells, myeloid leukemic cells, breast carcinoma cells, colon carcinoma cells, endometrial carcinoma cells, lung carcinoma cells, 10 ovarian carcinoma cells, prostate carcinoma cells, cervical carcinoma cells, osteosarcoma cells, testicular carcinoma cells and lymphoma cells.

15 In an embodiment of this invention in step (b) presence of the detectably labeled antibody 27.F7/Fab fragment bound to the surface of cells in the subject is detected by means for detecting the detectable label is an imaging device.

20 In an embodiment of this invention the imaging device is magnetic resonance imaging device.

In an embodiment of this invention the imaging device is X-ray immunoscintigraphy-imaging device.

25 ~~The present invention provides a method for monitoring progression of cancer, wherein cancer cells are TIP-2 antigen-bearing cancer cells, in a subject comprising: (a) administering to a subject diagnosed with cancer an antibody directed to an epitope on TIP-2 antigen or Fab fragment thereof, which epitope is recognized by monoclonal antibody 27.B1 produced by the hybridoma designated _____, said antibody/Fab fragment being detectably labeled, under appropriate conditions to bind the antibody to TIP-2 antigen on the surface of any cells in the subject; (b) determining presence of detectably labeled antibody 27.B1/Fab fragment bound to the surface of cells in the subject according to the according to the method of claim 23; (c) comparing the presence of detectably labeled~~

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antibody/Fab fragment 27.B1 bound to cells in step (b) with the presence of detectably labeled antibody 27.B1/Fab fragment bound to cells at (i) diagnosis time or (ii) after treatment, wherein a greater presence of detectably labeled antibody 27.B1/Fab fragment bound to cells in step (b) than at (i) diagnosis time or (ii) after treatment, indicates progression of the cancer in the subject and a lesser presence of detectably labeled antibody 27.B1/Fab fragment bound to cells in step (b) than at (i) diagnosis time or (ii) after treatment indicates regression of the cancer in the subject.

In an embodiment of this invention the detectable label is radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

In an embodiment of this invention the TIP-2 antigen-bearing cancer cells are human cancer cells.

In an embodiment of this invention the cancer cells are selected from a group consisting of melanoma cells, basal cell carcinoma cells, squamous cell carcinoma cells, neuroblastoma cells, glioblastoma multiforme cells, myeloid leukemic cells, breast carcinoma cells, colon carcinoma cells, endometrial carcinoma cells, lung carcinoma cells, ovarian carcinoma cells, prostate carcinoma cells, cervical carcinoma cells, osteosarcoma cells, testicular carcinoma cells and lymphoma cells.

In an embodiment of this invention in step (b) presence of the antibody 27.B1 bound to the surface of cells in the subject is detected by means for detecting the detectable label is an imaging device.

In an embodiment of this invention the imaging device is magnetic resonance imaging device.

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In an embodiment of this invention the imaging device is X-ray immunoscintigraphy-imaging device.

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The present invention provides a method for monitoring progression of cancer, wherein cancer cells are TIP-2 antigen-bearing cancer cells, in a subject comprising: (a) administering to a subject diagnosed with cancer an antibody directed to an epitope on TIP-2 antigen or an Fab fragment thereof, which epitope is recognized by monoclonal antibody 27.F7 produced by the hybridoma designated _____, said antibody/Fab fragment being detectably labeled, under appropriate conditions to bind the antibody to TIP-2 antigen on the surface of any cells in the subject; (b) determining quantity of detectably labeled antibody 27.F7/Fab fragment bound to the surface of cells in the subject according to the method of claim 23; (c) comparing the quantity of detectably labeled antibody 27.F7/Fab fragment bound to cells in step (b) with the presence of detectably labeled antibody 27.F7/Fab fragment bound to cells at (i) diagnosis time or (ii) after treatment, wherein a greater quantity of detectably labeled antibody 27.F7/Fab fragment bound to cells in step (b) than at (i) diagnosis time or (ii) after treatment, indicates progression of the cancer in the subject and a lesser quantity of detectably labeled antibody 27.F7/Fab fragment bound to cells in step (b) than at (i) diagnosis time or (ii) after treatment indicates regression of the cancer in the subject.

In the above described method, given the high heterogenicity of tumor cells, some cells may carry more of the antigen, some less. The quantity of the antigen may determine different stages of the disease, i.e. it may differentiate between a pre-cancerous lesions and a cancerous one.

In an embodiment of this invention the detectable label is radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

5 In an embodiment of this invention the TIP-2 antigen-bearing cancer cells are human cancer cells.

10 In an embodiment of this invention the cancer cells are selected from a group consisting of melanoma cells, basal cell carcinoma cells, squamous cell carcinoma cells, neuroblastoma cells, glioblastoma multiforme cells, myeloid leukemic cells, breast carcinoma cells, colon carcinoma cells, endometrial carcinoma cells, lung carcinoma cells, ovarian carcinoma cells, prostate carcinoma cells, cervical carcinoma cells, osteosarcoma cells, testicular carcinoma cells and lymphoma cells.

15 In an embodiment of this invention in step (b) quantity of the antibody 27.F7 bound to the surface of cells in the subject is detected by means for detecting the detectable label is an imaging device.

20 In the above-described embodiment of the invention, an estimate of accumulated quantity of the radionuclide-labeled antibody can be made by using an imaging device. Formulas assist in concluding whether the accumulation is specific or not.

25 In an embodiment of this invention the imaging device is magnetic resonance imaging device.

30 In an embodiment of this invention the imaging device is X-ray immunoscintigraphy-imaging device.

35 In an embodiment of this invention the TIP-2 antigen-bearing cancer cells are human cancer cells.

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In an embodiment of this invention the cancer cells are selected from a group consisting of melanoma cells, basal cell carcinoma cells, squamous cell carcinoma cells, neuroblastoma cells, glioblastoma multiforme cells, myeloid leukemic cells, breast carcinoma cells, colon carcinoma cells, endometrial carcinoma cells, lung carcinoma cells, ovarian carcinoma cells, prostate carcinoma cells, cervical carcinoma cells, osteosarcoma cells, testicular carcinoma cells and lymphoma cells.

The present invention provides a method for monitoring progression of cancer, wherein cancer cells are TIP-2 antigen-bearing cancer cells, in a subject comprising: (a) administering to a subject diagnosed with the cancer an antibody directed to an epitope on TIP-2 antigen or an Fab fragment thereof, which epitope is recognized by monoclonal antibody 27.B1 produced by the hybridoma designated _____, said antibody/Fab fragment being detectably labeled, under appropriate conditions to bind the antibody to TIP-2 antigen on the surface of any cells in the subject; (b) determining quantity of detectably labeled antibody 27.B1/Fab fragment bound to the surface of cells in the subject according to the method of claim 27; (c) comparing the quantity of detectably labeled antibody 27.B1/Fab fragment bound to cells in step (b) with the presence of detectably labeled antibody 27.B1 bound to cells at (i) diagnosis time or (ii) after treatment, wherein a greater quantity of detectably labeled antibody 27.B1/Fab fragment bound to cells in step (b) than at (i) diagnosis time or (ii) after treatment, indicates progression of the cancer in the subject and a lesser quantity of detectably labeled antibody 27.B1/Fab fragment bound to cells in step (b) than at (i) diagnosis time or (ii) after treatment indicates regression of the cancer in the subject.

In an embodiment of this invention the detectable label is radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

5 In an embodiment of this invention in step (b) quantity of the antibody 27.B1/Fab fragment bound to the surface of cells in the subject is detected by means for detecting the detectable label is an imaging device.

10 In an embodiment of this invention the imaging device is magnetic resonance imaging device.

In an embodiment of this invention the imaging device is X-ray immunoscintigraphy-imaging device.

15 In an embodiment of this invention the TIP-2 antigen-bearing cancer cells are human cancer cells.

20 In an embodiment of this invention the cancer cells are selected from a group consisting of melanoma cells, basal cell carcinoma cells, squamous cell carcinoma cells, neuroblastoma cells, glioblastoma multiforme cells, myeloid leukemic cells, breast carcinoma cells, colon carcinoma cells, endometrial carcinoma cells, lung carcinoma cells, 25 ovarian carcinoma cells, prostate carcinoma cells, cervical carcinoma cells, osteosarcoma cells, testicular carcinoma cells and lymphoma cells.

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30 The present invention provides a method for diagnosing cancer associated with the expression of TIP-2 antigen in a human subject which comprises: (a) obtaining mRNA from a sample of the subject's peripheral blood; (b) preparing cDNA from the mRNA from step (a); (c) amplifying DNA encoding TIP-2 antigen present in the cDNA prepared in step 35 (b) by a polymerase chain reaction utilizing at least two oligonucleotide primers, wherein each of the primers specifically hybridizes with DNA encoding TIP-2 antigen, wherein the primers comprise oligonucleotides having a

sequence included within the sequence of SEQ ID NO. __; and
(d) detecting the presence of any resulting amplified DNA,
the presence of such amplified DNA being diagnostic for
cancer associated with the expression of TIP-2 antigen.

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In the above described method, since the nucleic acid
structure of TIP-2 is known, one of skill in the art may
measure the expression of TIP-2 mRNA by Northern Blot since
the full mRNA sequence is known and the full size cDNA can
therefore be made. Another way to measure the expression
is by quantitative PCR using 18-21 mer primers on the basis
of the known mRNA sequence. One of skill in the art may
also synthesize specific primers or make the full size
cDNA. The full mRNA sequence of GIPC (GAIP Interacting
Protein, C terminus) is shown in Figure 24, with the part
corresponding to TIP-2 sequence underlined.

20

In an embodiment of this invention the presence of any
amplified DNA in step (d) is detected using a labeled
oligonucleotide probe which specifically hybridizes with
the amplified DNA.

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In an embodiment of this invention the labeled probe is
radiolabeled with ^{32}P or ^{33}P .

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The present invention provides a method for diagnosing
cancer associated with the expression of TIP-2 antigen in
a human subject which comprises: (a) obtaining mRNA from a
sample of the subject's peripheral blood; (b) preparing
cDNA from the mRNA from step (a);
(c) amplifying DNA encoding TIP-2 antigen present in the
cDNA prepared in step (b); (d) determining the amount of
any resulting amplified DNA; and (e) comparing the amount
of amplified DNA determined in step (d) with previously
determined standard amounts of amplified DNA, each standard
amount being indicative of a particular stage of cancer
associated with the expression of TIP-2 antigen.

In an embodiment of this invention the stage is precancerous cancer or benign dysplasia.

5 In an embodiment of this invention the cancer is selected from the group consisting of a tumor, cancer in the lymph nodes, and metastatic cancer.

10 The most widely used cancer staging system is the one based on the so-called TNM system (T, tumor; N, nodes; and M, metastases). Stage 0 amounts to Paget disease without a tumor or carcinom in situ with no lymph nodes involved and no metastases. Stage 1 is a tumor not larger that 2 cm without metastases or lymph nodes involved. Stage II is a tumor larger than 5 cm with auxillary lymph node(s) involvement, no distant metastases. Stage III is the same as Stage II with a string of the involved lymph nodes fixed to one another or to other structures and in the advance cases lymph nodes in mammary gland. Stage IV is the most advanced disease with a tumor of any -size, massive involvement of lymph nodes and any distant metastases.

15 20 As used herein, "whole TIP-2 antigen protein" comprises the amino acid sequence shown in Figure 23 (SEQ ID. NO. __).

25 The present invention further provides a vaccine comprising a monoclonal antibody produced by the method described herein and a suitable carrier.

30 The present invention also provides a vaccine comprising an effective amount of a monoclonal antibody of this invention and a pharmaceutically acceptable carrier.

35 According to certain embodiments of this invention, the condition is cancer and the amount of monoclonal antibody is sufficient to inhibit the growth of or eliminate the cancer. According to certain embodiments, the cancer is breast cancer, thyroid cancer or prostate cancer. According to certain embodiments, the condition is an

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infection and the amount of monoclonal antibody is sufficient to inhibit the growth of or kill the infectious agent. According to certain embodiments, the infectious agent is Hanta virus, HTLV I, HTLV II, HIV, herpes virus, influenza virus, Ebola virus, human papilloma virus, Staphylococcus, Streptococcus, Klebsiella, E. coli, anthrax or cryptococcus. According to certain embodiments, the condition is associated with a toxin and the amount of monoclonal antibody is sufficient to reduce the amount of or destroy the toxin. According to certain embodiments, the toxin is tetanus, anthrax, botulinum, snake venom or spider venom. According to certain embodiments, the condition is an autoimmune disease and the amount of monoclonal antibody is sufficient to reduce the amount of or destroy the offending antibody. In certain embodiments of this invention, the autoimmune disease is lupus, thyroiditis, graft versus host disease, transplantation rejection or rheumatoid arthritis.

According to certain embodiments of this invention, the monoclonal antibody is coupled to an effector molecule. According to another embodiment of this invention, the effector molecule is a cytotoxic agent, drug, enzyme, dye, or radioisotope. In another embodiment of this invention, the monoclonal antibody is coupled to a carrier. According to another embodiment of this invention, the carrier is a liposome.

The present invention further provides a method of treating a condition in a subject comprising administering to the subject an amount of the above-described vaccine effective to bind the antigen associated with the condition, thereby treating the condition in the subject.

The present invention further provides a method of preventing a condition in a subject comprising

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administering to the subject an amount of the above-described vaccine effective to bind the antigen associated with the condition, thereby preventing the condition in the subject. In an embodiment of the invention, the subject
5 previously exhibited the condition. In another embodiment of the invention, the vaccine is administered to a second subject.

10 According to an embodiment of the invention, the condition is associated with a cancer, a tumor, a toxin, an infectious agent, an enzyme dysfunction, a hormone dysfunction, an autoimmune disease, an immune dysfunction, a viral antigen, a bacterial antigen, a eukaryotic antigen,
15 or rejection of a transplanted tissue. In another embodiment of the invention, the condition is septicemia, sepsis, septic shock, viremia, bacteremia or fungemia. According to another embodiment of the invention, the cancer is thyroid cancer, breast cancer or prostate cancer.
20 In another embodiment of the invention, the infectious agent is Hanta virus, HTLV I, HTLV II, HIV, herpes virus, influenza virus, Ebola virus, human papilloma virus, Staphylococcus, Streptococcus, Klebsiella, E. coli, anthrax or cryptococcus. According to another embodiment of the invention, the toxin is tetanus, anthrax, botulinum, snake
25 venom or spider venom. In a further embodiment of the invention, the tumor is benign. In yet another embodiment of the invention, the enzyme dysfunction is hyperactivity or overproduction of the enzyme. According to a further embodiment of the invention, the hormone dysfunction is
30 hyperactivity or overproduction of the hormone. In another embodiment of the invention, the immune dysfunction is CD3 or CD4 mediated. In a further embodiment of the invention, the autoimmune disease is lupus, thyroiditis, graft versus
35 host disease, transplantation rejection or rheumatoid arthritis.

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The present invention further provides a vaccine comprising a whole TIP-2 antigen protein or a peptide form of TIP-2 and a suitable carrier.

5 The present invention also provides a vaccine comprising an effective amount of a whole TIP-2 antigen protein or a peptide form of TIP-2 and a pharmaceutically acceptable carrier.

10 According to certain embodiments of this invention, the condition is cancer and the amount of whole TIP-2 antigen protein or a peptide form of TIP-2 is sufficient to inhibit the growth of or eliminate the cancer. According to
15 certain embodiments, the cancer is breast cancer, thyroid cancer or prostate cancer. According to certain embodiments, the condition is an infection and the amount of monoclonal antibody is sufficient to inhibit the growth of or kill the infectious agent. According to certain
20 embodiments, the infectious agent is Hanta virus, HTLV I, HTLV II, HIV, herpes virus, influenza virus, Ebola virus, human papilloma virus, Staphylococcus, Streptococcus, Klebsiella, E. coli, anthrax or cryptococcus. According to certain embodiments, the condition is associated with a toxin and the amount of monoclonal antibody is sufficient
25 to reduce the amount of or destroy the toxin. According to certain embodiments, the toxin is tetanus, anthrax, botulinum, snake venom or spider venom. According to certain embodiments, the condition is an autoimmune disease and the amount of monoclonal antibody is sufficient to
30 reduce the amount of or destroy the offending antibody. In certain embodiments of this invention, the autoimmune disease is lupus, thyroiditis, graft versus host disease, transplantation rejection or rheumatoid arthritis.

35 According to certain embodiments of this invention, the whole TIP-2 antigen protein or peptide form of TIP-2 is coupled to an effector molecule. According to another

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embodiment of this invention, the effector molecule is a cytotoxic agent, drug, enzyme, dye, or radioisotope. In another embodiment of this invention, the monoclonal antibody is coupled to a carrier. According to another
5 embodiment of this invention, the carrier is a liposome.

The present invention further provides a method of treating a condition in a subject comprising administering to the subject an amount of the above-described vaccine effective
10 to bind the antigen associated with the condition, thereby treating the condition in the subject.

The present invention further provides a method of preventing a condition in a subject comprising
15 administering to the subject an amount of the above-described vaccine effective to bind the antigen associated with the condition, thereby preventing the condition in the subject. In an embodiment of the invention, the subject previously exhibited the condition. In another embodiment
20 of the invention, the vaccine is administered to a second subject.

According to an embodiment of the invention, the condition is associated with a cancer, a tumor, a toxin, an
25 infectious agent, an enzyme dysfunction, a hormone dysfunction, an autoimmune disease, an immune dysfunction, a viral antigen, a bacterial antigen, a eukaryotic antigen, or rejection of a transplanted tissue. In another embodiment of the invention, the condition is septicemia, sepsis, septic shock, viremia, bacteremia or fungemia.
30 According to another embodiment of the invention, the cancer is thyroid cancer, breast cancer or prostate cancer. In another embodiment of the invention, the infectious agent is Hanta virus, HTLV I, HTLV II, HIV, herpes virus,
35 influenza virus, Ebola virus, human papilloma virus, Staphylococcus, Streptococcus, Klebsiella, E. coli, anthrax

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or cryptococcus. According to another embodiment of the invention, the toxin is tetanus, anthrax, botulinum, snake venom or spider venom. In a further embodiment of the invention, the tumor is benign. In yet another embodiment of the invention, the enzyme dysfunction is hyperactivity or overproduction of the enzyme. According to a further embodiment of the invention, the hormone dysfunction is hyperactivity or overproduction of the hormone. In another embodiment of the invention, the immune dysfunction is CD3 or CD4 mediated. In a further embodiment of the invention, the autoimmune disease is lupus, thyroiditis, graft versus host disease, transplantation rejection or rheumatoid arthritis.

The present invention further provides a vaccine comprising dendritic cells which have been removed from a patient and contacted with a whole TIP-2 antigen protein or a peptide form of TIP-2 and a suitable carrier.

The present invention also provides a vaccine comprising an effective amount of dendritic cells which have been removed from a patient and contacted with a whole TIP-2 antigen protein or a peptide form of TIP-2 and a pharmaceutically acceptable carrier.

According to certain embodiments of this invention, the condition is cancer and the amount of dendritic cells which have been removed from a patient and contacted with whole TIP-2 antigen protein or a peptide form of TIP-2 is sufficient to inhibit the growth of or eliminate the cancer. According to certain embodiments, the cancer is breast cancer, thyroid cancer or prostate cancer. According to certain embodiments, the condition is an infection and the amount of monoclonal antibody is sufficient to inhibit the growth of or kill the infectious agent. According to certain embodiments, the infectious agent is Hanta virus, HTLV I, HTLV II, HIV, herpes virus,

influenza virus, Ebola virus, human papilloma virus, Staphylococcus, Streptococcus, Klebsiella, E. coli, anthrax or cryptococcus. According to certain embodiments, the condition is associated with a toxin and the amount of monoclonal antibody is sufficient to reduce the amount of or destroy the toxin. According to certain embodiments, the toxin is tetanus, anthrax, botulinum, snake venom or spider venom. According to certain embodiments, the condition is an autoimmune disease and the amount of monoclonal antibody is sufficient to reduce the amount of or destroy the offending antibody. In certain embodiments of this invention, the autoimmune disease is lupus, thyroiditis, graft versus host disease, transplantation rejection or rheumatoid arthritis.

According to certain embodiments of this invention, the dendritic cells which have been removed from a patient and contacted with whole TIP-2 antigen protein or peptide form of TIP-2 is coupled to an effector molecule. According to another embodiment of this invention, the effector molecule is a cytotoxic agent, drug, enzyme, dye, or radioisotope. In another embodiment of this invention, the monoclonal antibody is coupled to a carrier. According to another embodiment of this invention, the carrier is a liposome.

The present invention further provides a method of treating a condition in a subject comprising administering to the subject an amount of the above-described vaccine effective to bind the antigen associated with the condition, thereby treating the condition in the subject.

The present invention further provides a method of preventing a condition in a subject comprising administering to the subject an amount of the above-described vaccine effective to bind the antigen associated with the condition, thereby preventing the condition in the

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subject. In an embodiment of the invention, the subject previously exhibited the condition. In another embodiment of the invention, the vaccine is administered to a second subject.

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According to an embodiment of the invention, the condition is associated with a cancer, a tumor, a toxin, an infectious agent, an enzyme dysfunction, a hormone dysfunction, an autoimmune disease, an immune dysfunction, a viral antigen, a bacterial antigen, a eukaryotic antigen, or rejection of a transplanted tissue. In another embodiment of the invention, the condition is septicemia, sepsis, septic shock, viremia, bacteremia or fungemia. According to another embodiment of the invention, the cancer is thyroid cancer, breast cancer or prostate cancer. In another embodiment of the invention, the infectious agent is Hanta virus, HTLV I, HTLV II, HIV, herpes virus, influenza virus, Ebola virus, human papilloma virus, Staphylococcus, Streptococcus, Klebsiella, E. coli, anthrax or cryptococcus. According to another embodiment of the invention, the toxin is tetanus, anthrax, botulinum, snake venom or spider venom. In a further embodiment of the invention, the tumor is benign. In yet another embodiment of the invention, the enzyme dysfunction is hyperactivity or overproduction of the enzyme. According to a further embodiment of the invention, the hormone dysfunction is hyperactivity or overproduction of the hormone. In another embodiment of the invention, the immune dysfunction is CD3 or CD4 mediated. In a further embodiment of the invention, the autoimmune disease is lupus, thyroiditis, graft versus host disease, transplantation rejection or rheumatoid arthritis.

A monoclonal antibody which specifically binds and forms a complex with TIP-2 antigen located on the surface of human cancer cells, wherein the monoclonal antibody binds to the

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same antigen as monoclonal antibody 27.B1 produced by hybridoma 27.B1 (ATCC Designation No. PTA-1599) or monoclonal antibody 27.F7 produced by hybridoma 27.F7 (ATCC Designation No. PTA-1598).

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A murine monoclonal antibody of claim 1.

A chimeric monoclonal antibody of claim 1.

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A humanized monoclonal antibody of claim 1.

A human monoclonal antibody of claim 1.

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A monoclonal antibody of claim 1 which binds to the same epitope as monoclonal antibody 27.B1.

The monoclonal antibody 27.B1 produced by hybridoma 27.B1 (ATCC Designation No. PTA-1599).

20

A hybridoma cell producing the monoclonal antibody of claim 1.

The hybridoma of claim 8 designated 27.B1 (ATCC Accession No. PTA-1599).

25

A monoclonal antibody of claim 1 labelled with a detectable marker.

30

A monoclonal antibody of claim 10, wherein the detectable marker is a radioactive isotope, enzyme, dye, biotin, fluorescent label or chemiluminescent label.

A monoclonal antibody of claim 1 conjugated to a therapeutic agent.

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A monoclonal antibody of claim 12, wherein the therapeutic agent is a radioisotope, toxin, toxoid or chemotherapeutic agent.

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A monoclonal antibody of claim 1 conjugated to an imaging agent.

5 The monoclonal antibody of claim 14, wherein the imaging agent is a radioisotope.

A monoclonal antibody of claim 1 which binds to the same epitope as monoclonal antibody 27.F7.

10 The monoclonal antibody 27.F7 produced by hybridoma 27.F7 (ATCC Designation No. 1598).

15 The hybridoma of claim 8, designated 27.F7 (ATCC Designation No. 1598).

A method of detecting TIP-2 antigen bearing cancer cells in a sample comprising:

20 a) contacting the sample with an antibody directed to an epitope on TIP-2 antigen, or an Fab fragment of an antibody directed to an epitope on TIP-2 antigen, which epitope is recognized by the antibody or the Fab fragment, said antibody or Fab fragment being detectably labeled, under appropriate conditions to produce an antibody/Fab fragment-antigen complex comprising the detectably labeled antibody or Fab fragment bound to any TIP-2 antigen on the surface of cells in the sample;

30 b) removing any labeled antibody/Fab fragment not bound in the antibody/Fab fragment-antigen complex formed in step (a); and

35 c) determining presence of the antibody/Fab fragment-antigen complex by detecting the label of the detectably labeled antibody, presence of

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antibody/Fab fragment-antigen complex indicating
TIP-2 antigen-bearing cancer cells in the sample.

5 The method of claim 19, wherein the detectable label is
selected from the group consisting of radioactive isotope,
enzyme, dye, biotin, a fluorescent label or a
chemiluminescent label.

10 The method of claim 19, wherein the TIP-2 antigen-bearing
cancer cells are human cancer cells.

15 The method of claim 19, wherein the cancer cells are
selected from a group consisting of melanoma cells, basal
cell carcinoma cells, squamous cell carcinoma cells,
neuroblastoma cells, glioblastoma multiforme cells, myeloid
leukemic cells, breast carcinoma cells, colon carcinoma
cells, endometrial carcinoma cells, lung carcinoma cells,
ovarian carcinoma cells, prostate carcinoma cells, cervical
carcinoma cells, osteosarcoma cells, testicular carcinoma
20 cells and lymphoma cells.

The method of claim 19, wherein the antibody is a
monoclonal antibody.

25 The method of claim 19, wherein the epitope is recognized
by monoclonal antibody 27.F7 produced by the hybridoma
designated 27.F7 (ATCC Designation No. PTA-1598) .

30 The method of claim 19, wherein the epitope is recognized
by monoclonal antibody 27.B1 produced by the hybridoma
designated 27.B1 (ATCC Designation No. PTA-1599) .

35 The method of claim 19, wherein the monoclonal antibody is
a human monoclonal antibody or a murine monoclonal
antibody.

The method of claim 19, wherein the sample is selected from
the group consisting of serum, plasma, saliva, tears,

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5 mucosal discharge, urine, peritoneal fluid, cerebrospinal fluid, lymphatic fluid, bone marrow, breast biopsy, tissue, lymph nodes, prostate tissue, tissues from breast and prostate metastases, culture media, and other tumors where TIP-2 can be an associated antigen.

The method of claim 19, wherein TIP-2 is concentrated from the sample by alcohol precipitation prior to step (a).

10 The method of claim 19, wherein the sample is culture media.

15 A method of detecting TIP-2 antigen bearing cancer cells in a sample comprising:

20 a) contacting the sample with an antibody directed to an epitope on TIP-2 antigen, or an Fab fragment of an antibody directed to an epitope on TIP-2 antigen, which epitope is recognized by the antibody or the Fab fragment under appropriate conditions to produce an antibody/Fab fragment-antigen complex comprising the antibody or Fab fragment bound to any TIP-2 antigen on the surface of cells in the sample;

25 b) removing any antibody/Fab fragment not bound in the antibody/Fab fragment-antigen complex formed in step (a);

30 c) contacting the antibody/Fab fragment-antigen complex of step (b) with a second antibody which specifically binds to the antibody/Fab fragment-antigen complex, said second antibody being detectably labeled, under appropriate conditions to permit the second labeled antibody to bind to the antibody/Fab fragment-antigen complex;

35

d) removing any second labeled antibody not bound to the antibody/Fab fragment-antigen complex product in (c); and

5 e) determining presence of the antibody/Fab fragment-antigen complex bound to the second labeled antibody by detecting the label of second antibody, presence of antibody/Fab fragment-antigen complex indicating TIP-2 antigen-bearing
10 human cancer cells in the sample.

The method of claim 30, wherein the detectable label is radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

15 The method of claim 30, wherein the TIP-2 antigen-bearing cancer cells are human cancer cells.

20 The method of claim 30, wherein the cancer cells are selected from a group consisting of melanoma cells, basal cell carcinoma cells, squamous cell carcinoma cells, neuroblastoma cells, glioblastoma multiforme cells, myeloid leukemic cells, breast carcinoma cells, colon carcinoma cells, endometrial carcinoma cells, lung carcinoma cells,
25 ovarian carcinoma cells, prostate carcinoma cells, cervical carcinoma cells, osteosarcoma cells, testicular carcinoma cells and lymphoma cells.

30 The method of claim 30, wherein the antibody is a monoclonal antibody.

The method of claim 30, wherein the epitope is recognized by monoclonal antibody 27.F7 produced by the hybridoma designated 27.F7 (ATCC Designation No. PTA-1598) .

35 The method of claim 30, wherein the epitope is recognized by monoclonal antibody 27.B1 produced by the hybridoma designated 27.B1 (ATCC Designation No. PTA-1599).

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The method of claim 30, wherein the monoclonal antibody is a human monoclonal antibody or a murine monoclonal antibody.

The method of claim 30, wherein the sample is selected from the group consisting of serum, plasma, saliva, tears, mucosal discharge, urine, peritoneal fluid, cerebrospinal fluid, lymphatic fluid, bone marrow, breast biopsy, tissue, lymph nodes, prostate tissue, tissues from breast and prostate metastases, culture media, and other tumors where TIP-2 can be an associated antigen.

The method of claim 30, where TIP-2 is concentrated from the sample by alcohol precipitation prior to step (a).

A method for diagnosing cancer in a subject by detecting TIP-2 antigen-bearing cancer cells which comprises:

- (a) obtaining a sample of the subject's peripheral blood;
- (b) contacting the sample with an antibody directed to an epitope on TIP-2 antigen or an Fab fragment thereof, which epitope is recognized by the antibody or an Fab fragment thereof, said antibody being detectably labeled, under appropriate conditions to produce an antibody/Fab fragment-TIP-2 antigen complex comprising the detectably labeled antibody bound to any TIP-2 antigen on the surface of cells in the sample;
- (c) removing any labeled antibody/Fab fragment not bound in the antibody/Fab fragment-TIP-2 antigen complex formed in step (b); and
- (d) determining presence of the antibody/Fab fragment-TIP-2 antigen complex by detecting the

label of the detectably labeled antibody, presence of antibody/Fab fragment-TIP-2 antigen complex indicating diagnosis of cancer in the subject.

5

The method of claim 40, wherein the detectable label is radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

10

The method of claim 40, wherein the subject is human.

15

The method of claim 40, wherein the cancer is human melanoma, basal cell carcinoma, squamous cell carcinoma, neuroblastoma, glioblastoma multiforme, myeloid leukemia, breast carcinoma, colon carcinoma, endometrial carcinoma, lung carcinoma, ovarian carcinoma, prostate carcinoma, cervical carcinoma, osteosarcoma, testicular carcinoma and lymphoma.

20

The method of claim 40, wherein the antibody is a monoclonal antibody.

25

The method of claim 40, wherein the epitope is recognized by monoclonal antibody 27.F7 produced by the hybridoma designated 27.F7 (ATCC Designation No. PTA-1598) .

30

The method of claim 84, wherein the epitope is recognized by monoclonal antibody 27.B1 produced by the hybridoma designated 27.B1 (ATCC Designation No. PTA-1599).

35

The method of claim 84, wherein the antibody is a human monoclonal antibody or a murine monoclonal antibody.

The method of claim 84, wherein the sample is selected from the group consisting of serum, plasma, saliva, tears, mucosal discharge, urine, peritoneal fluid, cerebrospinal fluid, lymphatic fluid, bone marrow, breast biopsy, tissue, lymph nodes, prostate tissue, tissues from breast and

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prostate metastases, culture media, and other tumors where TIP-2 can be an associated antigen.

5 The method of claim 84, wherein TIP-2 is concentrated from the sample by alcohol precipitation prior to step (a).

A method for diagnosing cancer in a subject by detecting TIP-2 antigen-bearing cancer cells which comprises:

- 10 a) obtaining a sample of the subject's peripheral blood;
- 15 b) contacting the sample with an antibody directed to an epitope on TIP-2 antigen or Fab fragment thereof, which epitope is recognized by monoclonal antibody/Fab fragment or Fab fragment thereof, under appropriate conditions to produce an antibody/Fab fragment-TIP-2 antigen complex comprising the antibody bound to any TIP-2 antigen on the surface of cells in the sample;
- 20 c) removing any antibody/Fab fragment not bound in the antibody/Fab fragment-TIP-2 antigen complex formed in step (b);
- 25 d) contacting the antibody/Fab fragment-TIP-2 antigen complex of step (c) with a second antibody which specifically binds to the antibody/Fab fragment-TIP-2 antigen complex, said second antibody being detectably labeled, under appropriate conditions to permit the second labeled antibody to bind to the antibody/Fab fragment-TIP-2 antigen complex;
- 30 e) removing any second labeled antibody not bound to the antibody/Fab fragment-TIP-2 antigen complex product in (d); and
- 35

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f) determining presence of the antibody/Fab fragment-TIP-2 antigen complex bound to the second labeled antibody by detecting the label of second antibody, presence of antibody/Fab fragment-TIP-2 antigen complex indicating diagnosis of cancer in the subject.

The method of claim 108, wherein the detectable label is radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

The method of claim 108, wherein the subject is human.

The method of claim 108, wherein the cancer is human melanoma, basal cell carcinoma, squamous cell carcinoma, neuroblastoma, glioblastoma multiforme, myeloid leukemia, breast carcinoma, colon carcinoma, endometrial carcinoma, lung carcinoma, ovarian carcinoma, prostate carcinoma, cervical carcinoma, osteosarcoma, testicular carcinoma and lymphoma.

The method of claim 108, wherein the antibody is a monoclonal antibody.

The method of claim 108, wherein the epitope is recognized by monoclonal antibody 27.F7 produced by the hybridoma designated 27.F7 (ATCC Designation No. PTA-1598) .

The method of claim 84, wherein the epitope is recognized by monoclonal antibody 27.B1 produced by the hybridoma designated 27.B1 (ATCC Designation No. PTA-1599).

The method of claim 108, wherein the antibody is a human monoclonal antibody or a murine monoclonal antibody.

The method of claim 108, wherein the sample is selected from the group consisting of serum, plasma, saliva, tears,

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mucosal discharge, urine, peritoneal fluid, cerebrospinal fluid, lymphatic fluid, bone marrow, breast biopsy, tissue, lymph nodes, prostate tissue, tissues from breast and prostate metastases, culture media, and other tumors where TIP-2 can be an associated antigen.

The method of claim 108, wherein TIP-2 is concentrated from the sample by alcohol precipitation prior to step (a).

An in vivo method for diagnosing cancer in a subject by detecting TIP-2 antigen-bearing cancer cells which comprises:

- a) administering to the subject an antibody directed to an epitope on TIP-2 antigen or Fab fragment thereof, which epitope is recognized by the antibody or the Fab fragment, said antibody being detectably labeled, under appropriate conditions to bind the antibody to TIP-2 antigen on the surface of any cells in the subject; and
- b) determining presence of the detectably labeled antibody bound to the surface of cells in the subject, presence of detectably labeled antibody bound to cells indicating diagnosis of cancer in the subject.

The method of claim 116, wherein the detectable label is radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

The method of claim 116, wherein the subject is human.

The method of claim 116, wherein the cancer is human melanoma, basal cell carcinoma, squamous cell carcinoma, neuroblastoma, glioblastoma multiforme, myeloid leukemia, breast carcinoma, colon carcinoma, endometrial carcinoma, lung carcinoma, ovarian carcinoma, prostate carcinoma,

cervical carcinoma, osteosarcoma, testicular carcinoma and lymphoma.

5 The method of claim 116, wherein the antibody is a monoclonal antibody.

10 The method of claim 120, wherein the epitope is recognized by monoclonal antibody 27.F7 produced by the hybridoma designated 27.F7 (ATCC Designation No. PTA-1598) .

15 The method of claim 120, wherein the epitope is recognized by monoclonal antibody 27.B1 produced by the hybridoma designated 27.B1 (ATCC Designation No. PTA-1599).

20 The method of claim 116, wherein the antibody is a human monoclonal antibody or a murine monoclonal antibody.

25 The method of claim 116, wherein in step (b) presence of the antibody or Fab fragment thereof bound to the surface of cells in the subject is detected wherein means for detecting the detectable label is an imaging device.

30 The method of claim 116, wherein the imaging device is magnetic resonance imaging device.

35 The method of claim 116, wherein the imaging device is X-ray immunoscintigraphy imaging device.

A method for delivering exogenous material to TIP-2 antigen-bearing cancer cells of a human subject comprising administering to the subject a liposome carrying a conjugate of the exogenous material, wherein an antibody or an Fab fragment of the antibody is coupled to the outer surface of the liposome to target delivery to the cancer cells.

The method of claim 138, herein the exogenous material is selected from the group consisting of anti-cancer drugs,

radioisotopes, toxins, antibiotics, prodrugs, enzymes, and chemotherapeutic compounds.

5 The method of claim 138, wherein the TIP-2 antigen-bearing cancer cells are human melanoma cells, basal cell carcinoma cells, squamous cell carcinoma cells, neuroblastoma cells, glioblastoma multiforme cells, myeloid leukemic cells, breast carcinoma cells, colon carcinoma cells, endometrial carcinoma cells, lung carcinoma cells, ovarian carcinoma cells, prostate carcinoma cells, cervical carcinoma cells, osteosarcoma cells, testicular carcinoma cells and lymphoma cells.

15 A method for treating cancer in a human subject by evoking a specific immune response which comprises administering to the subject a whole TIP-2 antigen protein or a peptide fragment of TIP-2 to the subject.

20 The method of claim 141, wherein the specific immune response is complement-dependent cytotoxicity of TIP-2 antigen-bearing cancer cells.

25 The method of claim 141, wherein the specific immune response is activation of natural killer cells towards TIP-2 antigen-bearing cancer cells.

30 The method of claim 141, wherein the peptide fragment of TIP-2 antigen comprises the amino acid sequence Lys Leu Leu Gly Gly Gln Ile Gly Leu (SEQ. ID NO.).

The method of claim 141, wherein the peptide fragment of TIP-2 antigen comprises the amino acid sequence Ser Leu Leu Gly Cys Arg His Tyr Glu Val (SEQ. ID No.).

35 A method for treating cancer in a human subject by inducing apoptosis of cancer cells which comprises administering to the subject a whole TIP-2 antigen protein or a peptide fragment of TIP-2 to the subject.

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A method for treating cancer in a human subject by evoking a specific immune response which comprises:

- a) removing dendritic cells from said subject;
- b) contacting the dendritic cells of step (a) with a whole TIP-2 antigen protein or a peptide fragment of TIP-2; and
- c) reintroducing the dendritic cells of step (b) into said subject.

of TIP-2 antigen comprises the amino acid sequence Lys Leu Leu Gly Gly Gln Ile Gly Leu (SEQ. ID NO.).

The method of claim 147, wherein the peptide fragment of TIP-2 antigen comprises the amino acid sequence Ser Leu Leu Gly Cys Arg His Tyr Glu Val (SEQ. ID No.).

The method of claim 147, wherein the specific immune response is complement-dependent cytolysis of TIP-2 antigen-bearing cancer cells.

The method of claim 147, wherein the specific immune response is activation of natural killer cells or macrophages towards TIP-2 antigen-bearing cancer cells.

The method of claim 147, wherein the specific immune response is the production of antibodies in the subject against the whole TIP-2 antigen protein or the peptide fragment of TIP-2.

A method for treating cancer in a human subject by inducing apoptosis of cancer cells which comprises administering a whole TIP-2 antigen protein or a peptide fragment of TIP-2 to the subject.

A method for treating cancer in a human subject by passive immunization which comprises administering an antibody directed to an epitope on TIP-2 antigen or a peptide fragment thereof.

The method of claim 154, wherein the antibody induces apoptosis of TIP-2 antigen bearing cells.

An isolated peptide having the amino acid sequence Lys Leu Leu Gly Gly Gln Ile Gly Leu (SEQ. ID No.).

An isolated peptide having the amino acid sequence Ser Leu Leu Gly Cys Arg His Tyr Glu Val (SEQ. ID No.).

A method for immunohistochemical screening of a tissue section from a tumor sample for the presence of TIP-2 antigen bearing cancer cells which comprises:

a) contacting the tissue section from the tumor sample with an antibody directed to an epitope on TIP-2 antigen or Fab fragment thereof, which epitope is recognized by the antibody or Fab fragment said antibody/Fab fragment being detectably labeled, under appropriate conditions to produce an antibody/Fab fragment-TIP-2 antigen complex comprising the detectably labeled antibody bound to any TIP-2 antigen on the surface of cells in the tissue section;

a) removing any labeled antibody/Fab fragment not bound in the antibody/Fab fragment-TIP-2 antigen complex formed in step (a); and

b) determining presence of the antibody/Fab fragment-TIP-2 antigen complex by detecting the label of the detectably labeled antibody, presence of antibody/Fab fragment-TIP-2 antigen

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complex indicating TIP-2 antigen-bearing human cancer cells in the sample.

5 The method of claim 158 wherein the tissue section is preserved freshly frozen tissue or formalin-fixed tissue.

10 The method of claim 158 wherein the detectable label is radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

The method of claim 158, wherein the TIP-2 antigen-bearing cancer cells are human cancer cells.

15 The method of claim 158, wherein the cancer cells are selected from a group consisting of melanoma cells, basal cell carcinoma cells, squamous cell carcinoma cells, neuroblastoma cells, glioblastoma multiforme cells, myeloid leukemic cells, breast carcinoma cells, colon carcinoma cells, endometrial carcinoma cells, lung carcinoma cells, 20 ovarian carcinoma cells, prostate carcinoma cells, cervical carcinoma cells, osteosarcoma cells, testicular carcinoma cells and lymphoma cells.

25 The method of claim 158, wherein the antibody is a monoclonal antibody.

30 The method of claim 120, wherein the epitope is recognized by monoclonal antibody 27.F7 produced by the hybridoma designated 27.F7 (ATCC Designation No. PTA-1598) .

The method of claim 120, wherein the epitope is recognized by monoclonal antibody 27.B1 produced by the hybridoma designated 27.B1 (ATCC Designation No. PTA-1599).

35 The method of claim 158, wherein the antibody is a human monoclonal antibody or a murine monoclonal antibody.

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A kit for detecting the presence of TIP-2 antigen-bearing cancer cells in a sample comprising:

- 5 a) a solid support having a plurality of covalently linked probes which may be the same or different, each probe of which comprises a monoclonal antibody directed to an epitope on TIP-2 antigen or Fab fragment thereof; and
- 10 b) a means for determining the presence of monoclonal antibody/Fab fragment-TIP-2 antigen complex.

15 The kit of claim 165, wherein the means for determining the presence of the monoclonal antibody/Fab fragment-TIP-2 antigen complex is a detectably labeled second antibody which specifically binds to the monoclonal antibody directed to the epitope on TIP-2 antigen.

20 The kit of claim 165, wherein the monoclonal antibody directed to the epitope on TIP-2 antigen is human monoclonal antibody 27.F7 directed to an epitope on TIP-2 antigen, which epitope is recognized by monoclonal antibody 27.F7 produced by the hybridoma designated 27.F7 (ATCC
25 Designation No. PTA-1598).

30 The kit of claim 165, wherein the monoclonal antibody directed to the epitope on TIP-2 antigen is human monoclonal antibody 27.B1 directed to an epitope on TIP-2 antigen, which epitope is recognized by monoclonal antibody 27.B1 produced by the hybridoma designated 27.B1 (ATCC
35 Designation No. PTA-1599).

The kit of claim 165, wherein the detectable label is
radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

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The kit of claim 165, wherein the TIP-2 antigen-bearing cancer cells are human cancer cells.

5 The kit of claim 165, wherein the cancer cells are selected from a group consisting of melanoma cells, basal cell carcinoma cells, squamous cell carcinoma cells, neuroblastoma cells, glioblastoma multiforme cells, myeloid leukemic cells, breast carcinoma cells, colon carcinoma cells, endometrial carcinoma cells, lung carcinoma cells, 10 ovarian carcinoma cells, prostate carcinoma cells, cervical carcinoma cells, osteosarcoma cells, testicular carcinoma cells and lymphoma cells.

15 The kit of claim 165, wherein the antibody is a monoclonal antibody.

The kit of claim 165, wherein the antibody is a human monoclonal antibody or a murine monoclonal antibody.

20 The kit of claim 165, wherein the sample is selected from the group consisting of serum, plasma, saliva, tears, mucosal discharge, urine, peritoneal fluid, cerebrospinal fluid, lymphatic fluid, bone marrow, breast biopsy, tissue, lymph nodes, prostate tissue, tissues from breast and 25 prostate metastases, culture media, and other tumors where TIP-2 can be an associated antigen.

The kit of claim 165, wherein the sample is culture media.

30 The kit of claim 165, wherein the sample is a tumor sample.

A method for detecting the presence of TIP-2 antigen in biological fluid comprising:

- 35 a) contacting a sample of the biological fluid with a antibody directed to an epitope on TIP-2 antigen or Fab fragment thereof, which epitope is recognized by the antibody or Fab fragment

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thereof, said antibody being detectably labeled, under appropriate conditions to produce an antibody/Fab fragment-TIP-2 antigen complex comprising the detectably labeled antibody bound to any TIP-2 antigen on the surface of cells in the sample;

- c) removing any labeled antibody not bound in the antibody/Fab fragment-TIP-2 antigen complex formed in step (a); and
- d) determining presence of the antibody/Fab fragment-TIP-2 antigen complex by detecting the label of the detectably labeled antibody, presence of antibody/Fab fragment-TIP-2 antigen complex indicating TIP-2 antigen-bearing human cancer cells in the biological fluid.

The method of claim 178, wherein the detectable label is radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

The method of claim 178, wherein the TIP-2 antigen-bearing cancer cells are human cancer cells.

The method of claim 178, wherein the cancer cells are selected from a group consisting of melanoma cells, basal cell carcinoma cells, squamous cell carcinoma cells, neuroblastoma cells, glioblastoma multiforme cells, myeloid leukemic cells, breast carcinoma cells, colon carcinoma cells, endometrial carcinoma cells, lung carcinoma cells, ovarian carcinoma cells, prostate carcinoma cells, cervical carcinoma cells, osteosarcoma cells, testicular carcinoma cells and lymphoma cells.

The method of claim 178, wherein the antibody is a monoclonal antibody.

The method of claim 120, wherein the epitope is recognized by monoclonal antibody 27.F7 produced by the hybridoma designated 27.F7 (ATCC Designation No. PTA-1598).

5 The method of claim 120, wherein the epitope is recognized by monoclonal antibody 27.B1 produced by the hybridoma designated 27.B1 (ATCC Designation No. PTA-1599).

10 The method of claim 178, wherein the antibody is a human monoclonal antibody or a murine monoclonal antibody.

15 The method of claim 178, wherein the biological fluid is selected from the group consisting of serum, plasma, saliva, tears, mucosal discharge, urine, peritoneal fluid, cerebrospinal fluid, and lymphatic fluid.

The method of claim 178, wherein TIP-2 is concentrated from the sample by alcohol precipitation prior to step (a).

20 The method of claim 178, wherein the biological fluid is culture media.

25 The method of claim 178, wherein the monoclonal antibody directed to the epitope on TIP-2 antigen is human monoclonal antibody 27.F7 directed to an epitope on TIP-2 antigen, which epitope is recognized by monoclonal antibody 27.F7 produced by the hybridoma designated _____.

30 The method of claim 178, wherein the monoclonal antibody directed to the epitope of TIP-2 antigen is a murine monoclonal antibody directed to an epitope on TIP-2 antigen.

35 The method of claim 178, wherein the TIP-2 antigen is present on TIP-2 antigen-bearing cancer cells in the biological fluid.

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126. A method for monitoring progression of cancer, wherein cancer cells are TIP-2 antigen-bearing cancer cells, in a subject comprising:

- 5 a) administering to a subject diagnosed with cancer an antibody directed to an epitope on TIP-2 antigen or Fab fragment thereof, which epitope is recognized the antibody or Fab fragment thereof, said antibody being detectably labeled, under appropriate conditions to bind the antibody to TIP-2 antigen on the surface of any cells in the subject;
- 10 b) determining presence of detectably labeled antibody/Fab fragment bound to the surface of cells in the subject;
- 15 c) comparing the presence of detectably labeled antibody/Fab fragment bound to cells in step (b) with the presence of detectably labeled antibody bound to cells at (i) diagnosis time or (ii) after treatment, wherein a greater presence of detectably labeled antibody/Fab fragment bound to cells in step (b) than at (i) diagnosis time or (ii) after treatment, indicates progression of the cancer in the subject and a lesser presence of detectably labeled antibody/Fab fragment bound to cells in step (b) than at (i) diagnosis time or (ii) after treatment indicates regression of the cancer in the subject.
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The method of claim 209, wherein the detectable label is radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

The method of claim 209, wherein the TIP-2 antigen-bearing cancer cells are human cancer cells.

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The method of claim 209, wherein the cancer cells are selected from a group consisting of melanoma cells, basal cell carcinoma cells, squamous cell carcinoma cells, neuroblastoma cells, glioblastoma multiforme cells, myeloid leukemic cells, breast carcinoma cells, colon carcinoma cells, endometrial carcinoma cells, lung carcinoma cells, ovarian carcinoma cells, prostate carcinoma cells, cervical carcinoma cells, osteosarcoma cells, testicular carcinoma cells and lymphoma cells.

The method of claim 209, wherein the antibody is a monoclonal antibody.

The method of claim 120, wherein the epitope is recognized by monoclonal antibody 27.F7 produced by the hybridoma designated 27.F7 (ATCC Designation No. PTA-1598) .

The method of claim 120, wherein the epitope is recognized by monoclonal antibody 27.B1 produced by the hybridoma designated 27.B1 (ATCC Designation No. PTA-1599) .

The method of claim 209, wherein the antibody is a human monoclonal antibody or a murine monoclonal antibody.

The method of claim 209, wherein in step (b) presence of the detectably labeled antibody/Fab fragment bound to the surface of cells in the subject is detected by means for detecting the detectable label is an imaging device.

The method of claim 209, wherein the imaging device is magnetic resonance imaging device.

The method of claim 209, wherein the imaging device is X-ray immunoscintigraphy-imaging device.

A method for diagnosing cancer associated with the expression of TIP-2 antigen in a human subject which comprises:

- 5 (a) obtaining mRNA from a sample of the subject's peripheral blood;
- (b) preparing cDNA from the mRNA from step (a);
- 10 (c) amplifying DNA encoding TIP-2 antigen present in the cDNA prepared in step (b) by a polymerase chain reaction utilizing at least two oligonucleotide primers, wherein each of the primers specifically hybridizes with DNA encoding TIP-2 antigen, wherein the primers comprise oligonucleotides having a sequence included within the sequence of SEQ ID NO. __; and
- 15 (d) detecting the presence of any resulting amplified DNA, the presence of such amplified DNA being diagnostic for cancer associated with the expression of TIP-2 antigen.
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The method of claim 249, wherein the presence of any amplified DNA in step (d) is detected using a labeled oligonucleotide probe which specifically hybridizes with the amplified DNA.

25

The method of claim 249, wherein the labeled probe is radiolabeled with ³²P or ³³P.

30

A method for diagnosing cancer associated with the expression of TIP-2 antigen in a human subject which comprises:

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- (a) obtaining mRNA from a sample of the subject's peripheral blood;

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- (b) preparing cDNA from the mRNA from step (a);
 - (c) amplifying DNA encoding TIP-2 antigen present in the cDNA prepared in step (b);
 - (d) determining the amount of any resulting amplified DNA; and
 - (e) comparing the amount of amplified DNA determined in step (d) with previously determined standard amounts of amplified DNA, each standard amount being indicative of a particular stage of cancer associated with the expression of TIP-2 antigen.
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15 The method of claim 252, wherein the stage is precancerous cancer or benign dysplasia.

The method of claim 252, wherein the cancer is a tumor, cancer in the lymph nodes, or metastatic cancer.

20 A composition which comprises a suitable carrier and an effective amount of a monoclonal antibody, which monoclonal antibody is produced by a method comprising:

- 25
- (a) fusing a lymphoid cell capable of producing antibody with a trioma cell which does not produce any antibody and is obtained by fusing a heteromyeloma cell which does not produce any antibody with a human lymphoid cell so as to thereby form tetroma cells;
 - (b) incubating the tetroma cells formed in step (a) under conditions permissive for the production of antibody by the tetroma cells, so as to thereby produce the monoclonal antibody; and
 - (c) recovering the monoclonal antibody so produced.
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The composition of claim 79, wherein the monoclonal antibody is specific for an antigen associated with a condition in a subject.

5 The composition of claim 80, wherein the condition is cancer and the amount of monoclonal antibody is sufficient to inhibit the growth of or eliminate the cancer.

10 The composition of claim 81, wherein the cancer is breast cancer, thyroid cancer or prostate cancer.

15 The composition of claim 80, wherein the condition is an infection and the amount of monoclonal antibody is sufficient to inhibit the growth of or kill the infectious agent.

20 The composition of claim 83, wherein the infectious agent is Hanta virus, HTLV I, HTLV II, HIV, herpes virus, influenza virus, Ebola virus, human papilloma virus, Staphylococcus, Streptococcus, Klebsiella, E. coli, anthrax or cryptococcus.

25 The composition of claim 80, wherein the condition is associated with a toxin and the amount of monoclonal antibody is sufficient to reduce the amount of or destroy the toxin.

30 The composition of claim 85, wherein the toxin is tetanus, anthrax, botulinum, snake venom or spider venom.

35 The composition of claim 80, wherein the condition is an autoimmune disease and the amount of monoclonal antibody is sufficient to reduce the amount of or destroy the offending antibody.

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5 The composition of claim 80, wherein the monoclonal antibody is coupled to an effector molecule.

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The method of claim 93 or 94 wherein the condition is associated with a cancer, a tumor, a toxin, an infectious agent, an enzyme dysfunction, a hormone dysfunction, an autoimmune disease, an immune dysfunction, a viral antigen, a bacterial antigen, a eukaryotic antigen, or rejection of a transplanted tissue.

The method of claim 96, wherein the condition is septicemia, sepsis, septic shock, viremia, bacteremia or fungemia.

5 The method of claim 96, wherein the cancer is thyroid cancer, breast cancer or prostate cancer.

10 The method of claim 96, wherein the infectious agent is Hanta virus, HTLV I, HTLV II, HIV, herpes virus, influenza virus, Ebola virus, human papilloma virus, Staphylococcus, Streptococcus, Klebsiella, E. coli, anthrax or cryptococcus.

15 The method of claim 96, wherein the toxin is tetanus, anthrax, botulinum, snake venom or spider venom.

The method of claim 96, wherein the tumor is benign.

20 The method of claim 96, wherein the enzyme dysfunction is hyperactivity or overproduction of the enzyme.

The method of claim 96, wherein the hormone dysfunction is hyperactivity or overproduction of the hormone.

25 The method of claim 96, wherein the immune dysfunction is CD3 or CD4 mediated.

30 The method of claim 96, wherein the autoimmune disease is lupus, thyroiditis, graft versus host disease, transplantation rejection or rheumatoid arthritis.

35 The composition of claim 79, wherein the heteromyeloma cell is the cell designated B6B11 (ATCC accession number HB-12481).

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The composition of claim 79, wherein the heteromyeloma cell is a B6B11-like cell.

5 The composition of claim 79, wherein the human lymphoid cell is a myeloma cell.

The composition of claim 79, wherein the human lymphoid cell is a splenocyte or a lymph node cell.

10 The composition of claim 79, wherein the trioma cell is the cell designated MFP-2 (ATCC accession number HB-12482).

15 This invention will be better understood from the Experimental Details which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims which
20 follow thereafter.

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Experimental Details:

FIRST SERIES OF EXPERIMENTS

5 **EXAMPLE 1:** Construction of mouse-human heteromyeloma for
the production of human monoclonal antibodies.

Introduction

10 B6B11 or B6B11-like cells may be produced by the fusion of
mouse myeloma cells with human myeloma cells selected for
non-secretion of antibody. The specific generation
and application of heteromyeloma B6B11, is described herein
below. B6B11 was obtained by fusing the mouse HAT-
15 sensitive and G-418 resistant myeloma X63.Ag8.653 with the
subclone of human myeloma RPMI 8226 selected for non
secretion of lambda light chains. Fusion of human
splenocytes and B6B11 cells resulted in a fusion frequency
of 30-50 hybrids per 10^7 cells. This is similar to the
frequency of murine hybridoma formation. The hybrids are
20 readily cloned by limiting dilution, produce antibodies for
at least 10 month and grow in serum-free media. Two clones
were obtained which secreted human IgM reactive against
lipopolysaccharide (LPS) of Gram-negative bacteria. These
clones were obtained by fusing in vitro immunized human
25 splenocytes with the B6B11 cells. Anti-lipid A murine mAb
is known to prevent development of septic shock (Shnyra AA,
et al., 1990). Human mAbs have important clinical
applications.

30 **Results**

Heteromyeloma B6B11. Heteromyeloma, B6B11, was generated
by PEG-fusion of mouse myeloma 653 (HAT-sensitive, G-418)
with human RPMI 8226, which was selected for non-secretion
of lambda chains. Hybrids were selected in the presence of
35 HAT and G-418. Selection for 8-Ag resistance was done by
gradually increasing the 8-Ag concentration from 2 ug/ml to

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20 ug/ml for 2.5-3 weeks. The HAT-sensitive hybrid population 653x8226 was twice cloned. Clones were tested for the ability to produce hybrids with human lymphocytes. One clone, designated as B6B11, was selected. B6B11 cells died in medium containing aminopterin, during a period of 5-6 days; no revertants were detected for more than 18 months. In RPMI 1640 supplemented with 10% fetal calf serum (FCS), the line had the doubling time of about 25-30 hours, the maximal density in 75 cm² flasks was approximately 1.5×10^6 cells/ml (in a volume of 30 ml). B6B11 culture medium was tested for the presence of human immunoglobulin by enzyme linked immunoassay (ELISA) using rabbit anti-human immunoglobulin. B6B11 exhibited secretion of IgG, IgM or IgA. Staining the cell preparations with MAH-L,H by PAP-technique detected no traces of cytoplasmic light and heavy chain human immunoglobulin.

Karyotyping. Figure 1 illustrates the distribution of parental and B6B11 cells by chromosomal content. Chromosomal analysis of the heteromyeloma cells indicated that chromosomal number varies from 60 to 82.

Figure 2 shows a fragment of the G-banded karyotype of B6B11 cells. Normal mouse chromosomes constitute about 84% of the karyotype. There are several rearranged chromosomes. There are some markers for mouse myeloma chromosomes as well as rearranged heteromyeloma (human-mouse chimeric) chromosomes. One large telocentric chromosome was represented in all B6B11 metaphase plates examined. This suggested that the proximal portion of this chromosome contains mouse and the distal portion contains human genetic material of chromosome 3 (3p21.1-3p ter). Localization of human material was performed as described (33). In some of analyzed B6B11, cells human chromosome 19 and human chromosome 7 was deleted.

Fusion Of B6B11 Cells With Human Lymphocytes. Fusion of B6B11 cells with freshly isolated peripheral blood lymphocytes (PBL) and splenic lymphocytes (SPL) was performed as described herein below in the Experimental Procedures Section. Fusion of peripheral blood lymphocytes (PBL) and pokeweed mitogen (PWM) treated peripheral blood lymphocytes (PBL) resulted in low hybridoma yield (1-5 hybrids per 10^7 lymphocytes), while fusion with splenic lymphocytes (SPL) and pokeweed mitogen (PWM) treated splenic lymphocytes (SPL) yielded 30-60 hybrids per 10^7 cells (see Table 1). After the fusion, cells were seeded at a density of 1.5×10^5 cells per well. Variations in the cell ratios of 1:1 to 1:2 (heteromyeloma:lymphocyte) had no effect on the fusion efficiency for PBL or SPL. However, fusion efficiency was dramatically reduced at B6B11: lymphocyte ratios of 1:4 to 1:8.

Table 1

Fusion of human lymphocytes with B6B11 cells.

	LYMPHOCYTES			
	PBL	PBL-PWM	SPL	SPL-PWM
Number of fusion	4	6	10	8
Number of wells	1536	2304	4800	3072
Growth ² , %	4	6, 9	55	72
Hybrid populations ³ per 10^7 lymphocytes	1-3	3-5	30-50	40-60
Wells with Ig secretion ⁴ , %	95	92	84	82

¹ Fresh isolated peripheral blood lymphocytes (PBL) and splenocytes (SPL) were activated with PWM (5 ug/ml) for 7-9 days in complete RPMI 1640 supplemented with 15% FCS.

² Wells with hybrids (% of the total well number)

³ After fusion cells were seeded at a density of 15×10^4 cells/well

- ⁴ Total Ig production was determined by ELISA with mouse monoclonal antibodies to H- and L-chains of human Ig

The effects of splenocyte stimulation with various mitogens on the fusion efficiency are illustrated in Figure 3. PWM treatment significantly increased the efficiency of SPL hybridization compared with ConA-treatment, PHA-treatment, LPS-treatment or untreated SPL. Fusion efficiency was dependent on the timing of the HAT addition. When HAT was added immediately following fusion, the yield decreased to 10-15 hybrids per 10^7 lymphocytes (for SPL).

Cloning of hybrids with SPL and PBL (stimulated and non-stimulated) indicated that PBL could not be used for hybridoma formation. Cloning was performed 4-6 weeks after fusion in 50% epithelial conditioned media (ECM) (pre-incubated for 24 hours at 37°C in 96-well plates) and 50% RPMI 1640 containing 15% FCS. Results were determined at in 2-2.5 weeks. Cloning efficiency (1.5-2 cells per well) was 50-80% for SPL and 10-30% for PBL. ELISA using rabbit anti-human immunoglobulin and MAH-L,H indicated that the total immunoglobulin production was present in 90-95% of growing hybrids with PBL and 80-85% with SPL hybridomas. Based on SPL was selected for PWM stimulation and in vitro immunization.

In order to increase the efficiency of hybridization, splenocytes were treated with 2.5 mM Leu-Ome and fused with B6B11 cells at ratio of 1:1 or 1:2 (B6B11: SPL) (see Table 2). The effect on this treatment was apparent after 18-24 hours of cultivation with PWM; SPL without Leu-Ome treatment exhibited blasts only after three days. The efficiency of hybridization of Leu-Ome-treated SPL was somewhat higher (80%) compared with non-treated SPL (72%). This treatment considerably increased (93%) the number of Ig-secreting hybrids.

Table 2

Effect of Leu-Ome treatment of splenocytes on the efficiency of their hybridization with B6B11 cells (data from 3 spleens)

Lymphocytes	Number of wells	Wells with hybrid populations, (%)	Wells2 with Ig secretion, (%)
SPL	1440	1034 (72)	825 (80)
SPL-Leu-Ome	864	691 (80)	642 (93)

¹ Splenocytes were isolated in LSM. One portion was treated with Leu-Ome (2.5 mM, 40 minutes in serum-free RPMI 1640), the other served as a control. Prior to fusion both portions were cultured for 7 days in complete RPMI 1640 supplemented with 15% FCS in the presence of 5 µg/ml PWM.

² Ig production was determined by ELISA with mouse monoclonal antibodies to H- and L-chains of human Ig.

The heteromyeloma cells were fused with Leu-Ome-treated splenocytes immunized with Salmonella minnesota Re595 (Re595) in the presence of PWM and mouse thymocyte conditioned media (TCM) (Table 3). The hybridoma culture supernatants were tested for anti-bacterial antibodies at different stages of hybrid growth: (1) after transferring responding populations to 24-well plates and (2) after cloning and subsequent clonal expansion. Two independent clones producing anti-bacterial antibodies were selected. ELISA using immobilized lipopolysaccharide (LPS) or immobilized Re595 and LPS in solution determined that the antibodies produced by both clones reacted with LPS.

ELISA using immobilized Re595 monoclonal mouse anti-human isotypes and goat anti-mouse peroxidase conjugate absorbed with human immunoglobulin, determined that the antibody isotype was IgM-kappa. Both clones were adapted to serum free media (SFM) by gradual replacing of the growth medium containing 10% FCS. The maximal density upon culturing in SFM was approximately 1.2×10^6 cells/ml. SFM-adapted cells were cloned as described above. The efficiency and cloning

time were similar to those of the cells cultured in serum-supplemented RPMI 1640 medium.

Table 3

Fusion of in vitro immunized splenocytes¹ with B6B11 cells.

	Number of fusion		
	1	2	3
Number of wells	288	864	576
Wells with hybrid populations, (%)	193 (69)	734 (85)	472 (82)
Wells with ig secretion, (%)	173 (90)	675 (92)	420 (89)
Primary response ² to Re595, number of wells	9 (4.5)	---	17 (3.6)
Secondary response ³ , number of wells	2	---	16
Number of responding populations after cloning	---	---	2

¹ Splenocytes after treatment with Leu-Ome (2.5 mM, 40 min) were in vitro immunized with S.minnesota Re595 (10^7 - 10^{10} cells/ml) in the presence of PWM (5 ug/ml) and TCM for 7-9 days. Fusions with B6B11 cells were done at ratios 1:1 and 1:2

² ELISA of hybridoma culture supernatants from 96-well plates (rabbit anti-human Ig).

³ ELISA of hybridoma culture supernatants after transferring in 24-well plates (rabbit anti-human Ig).

DNA analysis. Figure 4 illustrates the distribution of the DNA content by parental lines, B6B11 heteromyeloma and B6B11-splenocyte hybrid. The DNA of heteromyeloma cells consists of 78.7% of the total parental DNA. The DNA content of B6B11-splenocyte hybrid cells is 3% greater than that of B6B11 cells.

Discussion

A partner cell line for production of human monoclonal antibodies was generated by somatic hybridization of mouse X63.Ag8.653 and human RPMI 1640 myeloma cells. Adaptation to medium with 8-Ag, subsequent cloning and selection by hybridization efficiency led to a heterohybrid clone which was designated B6B11. Fusion between heterohybrid lines and lymphocytes gives essentially stable productive hybrids (Raison RL, et al., 1982). The mechanisms underlying this phenomenon are unknown. It is suggested that human chromosomes or their fragments retained in the partner line after the first fusion modify the intracellular environment in such a way that the human lymphocyte chromosomes or fragments after the second fusion are stabilized (Oestberg L, and Pursch E., 1983). The large number of chromosomes, the presence of hybrid marker chromosomes and increased DNA content observed in the experiments described herein, confirmed the hybrid nature of B6B11 cells. The DNA content of B6B11-SPL hybrid cells was also increased. Immunocytochemical testing for intracellular heavy and light chains and ELISA testing for immunoglobulin secretion demonstrated that B6B11 cells produce neither immunoglobulins nor heavy and light chains. Fusion of B6B11 with SPL resulted in more hybrids than fusion with PBL (30-50 per 10^7 SPL compound to 1-5 per 10^7 PBL). Cloning efficiency with SPL was 50-80% as compared to 10-30% with PBL. Thus SPL were the more preferable partner for fusion. The culture media was conditioned by endothelial cells; which was deemed crucial for viability and clonogeneity of the hybrids. In the case of B6B11-PBL hybrids, immunoglobulin secretion was detected in up to 95% of the hybrids. To increase the yield of immunoglobulin-secreting hybrids after fusion with SPL (up to 93%) Leu-Ome was used. Almost all hybrids secreted antibodies of unknown specificity. The antibody production by B6B11 hybrids was stable for at least 10 months. The hybrids were readily adapted to serum-free media, thereby facilitating a ex-vivo antibody production.

Two antibody-producing clones (with probably similar specificity to LPS of S.minnesota Re595) were obtained after fusion of immunized SPL with B6B11 cells. As demonstrated herein, human-mouse heteromyeloma, B6B11, is useful for producing human monoclonal antibodies to various antigens. Proper in vitro sensitization of lymphocytes is also of critical importance for generating human antibodies.

Experimental Procedures

Cell Culture. 8-Azaguanine (8-Ag) resistant mouse myeloma X63.Ag8.653 (653) cells were transfected with plasmid pBgl-neoR (Dr. A. Ibragimov) as described below. The myeloma cells were maintained in DMEM medium supplemented with 10% fetal calf serum (FCS), 4 mM L-glutamine, 1 mM Sodium pyruvate, non-essential amino acids and vitamins (Flow Laboratories). Prior to fusion the cells were passaged 3 times in the presence of 20 μ g/ml 8-Ag (Sigma) and 500 μ g/ml G-418 (Gibco).

Human myeloma cell line RPMI 8226 (8226) was cultured in RPMI 1640 medium with above-mentioned supplements (regular RPMI 1640). The hybrid heteromyeloma B6B11 was cultured either in regular RPMI 1640 with 10% FCS or in serum-free media which represented 1:1 mixture of Iscove's modification of Dulbecco medium (IMDM) and HAM F-12 (Flow Laboratories) supplemented with bovine serum albumin fraction #5, 2 mg/ml, (BSA) (Sigma), bovine insulin, 5 μ g/ml (Serva), human transferrin, 5 μ g/ml (Sigma), progesterone, 6 ng/ml (Gibco), hydrocortisone, 60 ng/ml (Gibco). Hybridomas were adapted to this serum free medium (SFM) by gradual replacement of the growth medium containing 10% FCS. All cells were cultured in a humidified atmosphere of 5.5% CO₂/94.5% air at 37°C.

Human peripheral blood lymphocytes (PBL) were isolated using lymphocytes separation medium (LSM) (Flow Laboratories) as per manufacturer instructions. Spleens

collected at autopsy not later than 2 hours after death (males aged 50-60 years old) were homogenized and splenocytes (SPL) were isolated in LSM.

5 **Production of Geneticin (G-418) Resistant 653 Myeloma**

Cells. Cells were washed in sterile phosphate buffered saline (PBS) without Ca^{++} or Mg^{++} . pBgl-neoR Plasmid DNA linearized by BamH1 (constructed by P.Chumakov, Institute of Molecular Biology of the Academy of Sciences of the USSR, Moscow, USSR) was added to the cell suspension. Prior to adding the DNA to the cell suspension, the DNA was twice phenol extracted using phenol-ether at 4°C, 96% ethanol precipitated and dried under sterile conditions.

10
15 Transfection was performed by electroporation at 4°C using a unit constructed by L.Chernomordik (Institute of Electrical Chemistry of the Academy of Sciences of the USSR, Moscow, USSR). Approximately 4×10^6 653 myeloma cells and 3.5 μg of plasmid DNA were combined in an 80 μl electroporation chamber. The final concentration of DNA was 44 $\mu\text{g}/\text{ml}$). An electrical current impulse of 1.7 Kv/cm was pulsed through the chamber for 100 μsec . After resting for 10 minutes the cells were transferred to 0.5 ml complete media in 16 mm^2 wells at 5×10^3 and 2×10^4 cells/well. 20
25 After 36 hours, 0.5 ml of media containing 1 mg/ml of Geneticin (G-418) was added to a final concentration of 0.5 mg/ml. Subsequently, 50% of the media volume was changed every 2 days for 12 days.

30 **Production of heteromyeloma.** G-418-resistant 653 cells were mixed with 8226 cells at a 1:1 ratio and pelleted. 50% (v/v) polyethylenglycol (PEG) 3350 (Sigma) was added (200-300 μl per $4-5 \times 10^7$ cells) for 1 min with constant stirring. Several portions of serum-free RPMI 1640 (RPMI-S⁻) were added for 5 minutes (first 10 ml), 1 minute (10 ml), 35
and 1 minute (30 ml). Cells were pelleted resuspended in regular RPMI 1640 with 20% FCS, hypoxanthine (1×10^{-4} M),

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aminopterine (4×10^{-7} M), thymidine (1.6×10^{-5} M) (HAT, Flow Laboratories) and 500 $\mu\text{g/ml}$ G-418 and seeded in 96-well plates (Linbro) at a density of 10^5 cells per well. At two weeks the medium (1/2 volume) was replaced with medium containing hypoxanthine (2×10^{-4} M), thymidine (3.2×10^{-5} M) (HT, Flow laboratories) and G-418 (500 $\mu\text{g/ml}$). The procedure was repeated after two weeks.

Production of human monoclonal antibodies. Fusion of B6B11 cells with human lymphocytes was accomplished by the above-described method with following modifications. Lymphocytes were mixed with B6B11 at a 1:1 or a 1:2 ratio, pelleted, washed with RPMI 1640-S- and incubated with PEG (600 μl per 10^5 cells) for 3 minutes with constant stirring. The portions of added RPMI-S- were as follows: 10 ml/10 minutes, 10ml/10 5 minutes, 10 ml/1 minute. Cells were pelleted, re-suspended in regular RPMI supplemented with 15% FCS and seeded in 96-well plates (1.5×10^5 cells per well). HAT-medium was added after 24 hours. The growth medium (1/2 volume) was replaced with fresh HAT in 7-9 days. HAT-medium was replaced with HT-medium at 15-18 days.

Cloning. Parent heteromyeloma and hybridoma cells were cloned by the limiting dilution method in medium conditioned by human umbilical or aortic endothelial cells (Antonov AS, et al., 1986) (gift from Dr. A. Antonov) (ECM). 100 $\mu\text{l/well}$ was incubated in 96-well plates at 37°C overnight. Cells were planted at approximately 1 to 2 cells per well. The culture medium was tested for antibodies at 2.5-3 weeks.

Immunization in vitro. Freshly isolated lymphocytes were resuspended in RPMI-S- containing 2.5 mM L-leucine methyl ester (Leu-OMe) (Borrebaeck, CAK, et al., 1987) to a final concentration of 10^7 cells per ml. After 40 minutes of incubation at room temperature, cells were washed 3 times

with RPMI-S- and resuspended in regular RPMI 1640 supplemented with 15% FCS. Medium conditioned by mouse thymocytes (TCM) was used as a source of lymphokines (Reading CL., 1982). Pokeweed mitogen (PWM) (Flow laboratories) to a final concentration 5 μ g/ml, TCM (25%) and antigen in different concentrations were added to the cell suspension. The cell suspension ($4-6 \times 10^6$ cell/ml) was transferred to flasks (30 ml/75 cm² flask). Fusion was performed after 7-9 days of cultivation. Concanavalin A (ConA) (Flow 5-10 μ g/ml), Phytohemagglutinin (PHA) (Flow, 5-10 μ g/ml) and lipopolysaccharide (LPS) (SIGMA, 10-15 μ g/ml) were used instead of PWM. S.minnesota Re595 (gift of Dr. O. Luderitz, Max Plank Institute fur Immunologie, Feiburg, Germany) was used as an antigen. The bacteria were grown in medium containing 16 g/l tryptic soy broth (TSB), Difco), 16 g/l brain-heart infusion (BHI) (Difco) and 4 g/l yeast extract (YE) (DIFCO) for 18 hours at 37°C with constant stirring and then heat inactivated. The antigen concentration varied from 10^7-10^{10} cells/ml.

Determination of antibodies and non-specific Ig production.

Enzyme linked immunoassay (ELISA) was used to test hybridoma supernatants for the presence of antibodies against Salmonella minnesota Re595 and LPS.

Screening for mAbs reactive against bacteria. 96-well plates were covered with glutaraldehyde (1%, 100 μ l per well) for 2 hours at room temperature. The plates were washed with distilled water 3 times. Bacteria were resuspended in 50 mM ammonium carbonate buffer (pH 9.6) and transferred to plates (5×10^7 cells in 100 μ l per well), centrifuged at 780 x g for 30 minutes and washed with distilled water 4 times. The supernatants tested (100 μ l) were supplemented with 0.1% Tween 20 (Fluka), put into bacteria-containing wells and incubated for 1 hour at room temperature. The media was then removed and the wells were washed with distilled water. Affinity purified rabbit anti-human immunoglobulin conjugated to alkaline

phosphatase (RAH-AP), diluted in tris-buffered solution (TBS, 50 mM, pH 7.4), containing 0.1% Tween 20 was added to 1 μ g in 100 μ l per well. After 1 hour of incubation at room temperature and 6 washes with distilled water 100 μ l of 4-nitrophenyl-phosphate (1 mg/ml, Sigma) in diethanolamine buffer (10% diethanolamine, 0.5 mM MgCl₂, pH 9.8) was added. After 1 hour, the results were read using a Multiscan (Flow Laboratories) at 405 nm. The negative control was culture medium RPMI 1640 supplemented with 15% FCS.

Screening for mAbs reactive against lipopolysaccharide.

LPS was purified from Salmonella minnesota Re595 as described (Galanos G, et al., 1969). The LPS preparation was sonicated and transferred to the plates at 2.5 μ g per well in 5mM ammonium carbonate buffer (pH 9.6). After overnight incubation at room temperature, the above described procedures for determining mAb reactive against bacteria were performed.

Screening for non-specific production of mAbs.

Non-specific production of immunoglobulin and separate chains was assessed after the addition of 100 μ l of rabbit anti-human immunoglobulin (10 μ g/ml in phosphate buffer, PBS, pH 7.2) or 100 μ l/well (10 ng/ml in PBS) of mouse monoclonal antibodies to light and heavy chains of human immunoglobulin (MAH-L, H) (Rokhlin OV, 1989) (gift of O. Rochlin, CRC, Moscow). Subsequent procedures were performed as described above.

Determination of the isotype of secreted antibodies. The isotype of human antibodies was determined by ELISA using murine anti-human light and heavy chains (MAH-L, H) and goat anti-mouse immunoglobulin (25 μ g/ml) conjugated to peroxidase and absorbed with human immunoglobulin.

Determination of cytoplasmic light or heavy chains production.

Production of cytoplasmic light and/or heavy chains in hybridomas, B6B11 and the parental cell lines was estimated immunocytochemically using the peroxidase-anti-peroxidase system (PAP). Cell smears were air-dried, fixed for 45 seconds with 10% formaldehyde (v/v) and 45% acetone (v/v) in phosphate buffered saline (PBS, 10 mM NaH_2PO_4 , pH 6.6) and incubated with MAH-L,H (200 μl , 5-10 mg/ml). Then 1 ml rabbit anti-mouse immunoglobulin (38 mg/ml in PBS) was added. All incubations were 30 minutes. Washings were performed using PBS for 10 minutes.

Chromosomal analysis.

Preparations of metaphase chromosomes were obtained by the following technique. Colchicine was added to cells during exponential growth (1.5-2 hours to parental lines and B6B11 cells). Cells were then trypsinized and stained for G-banding as described (Seabright S., 1971) (10-15 plates from each line). To count chromosome number, at least 50 metaphase figures were analyzed for each cell line.

DNA analysis by flow cytometry.

To estimate the DNA content the cells (1×10^6) were fixed with 1 ml 70% ethanol, washed, incubated for 2-3 hours with 0.3 mg/ml Ribonuclease A (Serva) in Hank's solution (pH 7.4) and stained for 2 hours with propidium iodide (0.05 mg/ml, Sigma) in Hank's solution. The DNA content was measured in a FACS-II cytofluorometer (Becton Dickinson). Fluorescence was excited by an argon ion laser at 488 nm (164-05 Model, Spectra-Physics) at a power of 400 mW and registered behind a 600 nm long pass interference filter (Ditric Optica).

Parental lines.

The myeloma line 653 was maintained in DMEM supplemented with 10 FCS, 20 ng/ml 8-Azaguanine and 500 $\mu\text{g/ml}$ G-418. The myeloma line 8226 producing lambda chains of human Ig was cultured in RPMI-C containing 10% FCS. In order to create a heteromyeloma, a non-producing

clone of 8226 line was selected by cloning in ECM (2 cells per well). Lambda chain production was estimated at 2-2.5 weeks using MAH-L, H. The frequency of non-secreting clones was 1×10^{-3} .

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EXAMPLE 2: Trioma MFP-2, a fusion partner for generating Human Monoclonal antibodies.

Introduction

10 A precursor hybridoma cell line was obtained by hybridization of the commercially available human myeloma cell line RPMI 8226 and mouse myeloma X63.Ag8.653 resistant to both 8-Azaguanine (8-Ag) and Geneticin 418 (G-418). One of the resulting clones, B6B11, was selected in the
15 presence of G-418. B6B11 was grown in the presence of increased concentrations of 8-Ag and is resistant to both G-418 and 8-Ag (See Example 1).

20 Although B6B11 can be used to make human hybridomas by fusing with human lymph node-derived lymphocytes or spleen-derived lymphocytes, B6B11 was not capable of fusing with human peripheral blood lymphocytes (PBL) or resulted in a very low yield of hybrids (see example 1).

25 In order to overcome this problem, B6B11 was fused with human lymph node lymphocytes and several hybrids were obtained. The resulting cells were analyzed for human immunoglobulin production or production of separate immunoglobulin chains. Those clones, which did not
30 synthesize immunoglobulin or immunoglobulin chains were selected for further evaluation in terms of fusion capability and antibody secretion potential. These hybrids were determined to be quite stable. These fusion products were designated "modified fusion partner" (MFP) cells.
35 These MFP cells as the product of the fusion of the B6B11 hybridoma and lymphocytes are referred to herein as "trioma" cells because they are, in essence, the product of a three fused cells. One of the clones, MFP-2, exhibited a very

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high efficiency for fusing with peripheral blood lymphocytes as well as for fusing with human lymphocytes of any varied origin (i.e. lymph nodes, spleen, Peyer's patches etc). MFP-2 was selected on the basis of its superior characteristics and stability as a fusion partner and was used in the experiments described herein below.

The products of fusions between the MFP trioma cells and lymphocytes are referred to herein as "tetroma" cells because they are, in essence, the product of four fused cells.

Results

Immunoglobulin Production. In order to demonstrate that human hybrioma (trioma) fusion partner cell line, MFP-2, is capable of fusing with human lymphocytes and producing high yields of hybrids with stable immunoglobulin production, experiments were performed using human lymphocytes from different sources.

The heteromyeloma cell line, B6B11 (precursor to MFP-2), can be fused with high efficiency with lymph node and spleen lymphocytes. (See, Example 1). Up to 90% of the resulting hybrids produced IgG or IgM. However, B6B11 was incapable of fusing to lymphocytes derived from peripheral blood (PBLs). The trioma cell line, MFP-2, (resulting from a fusion between B6B11 and human lymph node lymphocytes) overcame this problem and exhibited high fusion efficiency with PBL, yielding a high rate of immunoglobulin production by the resulting tetroma hybrids. The capability of MFP-2 to fuse with PBL was tested in two ways: (1) by fusion with freshly isolated lymphocytes in suspension, and (2) by fusion with thawed lymphocytes which had been stored frozen for various periods of time. (See Experimental Procedures). The results of these experiments are shown in Figure 5.

The fusion efficiency was 10^5 (1 hybrid per 10^5 lymph-

mphocytes). Thirty primary hybridoma (tetroma) populations were obtained and analyzed for capacity to secrete immunoglobulin. (A primary hybridoma population is likely to be a mixture of two or more individual clones). Twenty-seven populations (90%) produced IgM at a level 5-fold greater than background. Twenty-four populations (80%) secreted IgE at a level 5-fold greater than background. The fusion of MFP-2 with lymphocyte suspensions which had been frozen and thawed also resulted in immunoglobulin-producing hybrids. Nineteen percent and 11% of these hybridoma populations produced human IgM and IgG respectively. The efficiency of fusion, itself, was not effected by the freeze-thaw procedure. These results demonstrate that both freshly isolated as well as frozen PBLs can be used to generate human hybridomas capable of producing antibody.

Identification of tumor-associated antigens and production of specific antibodies using the MFP-2 fusion partner:

Human monoclonal antibodies against thyroglobulin. In this experiment, human anti-thyroglobulin antibodies were generated by MFP-2 fusion using lymph nodes from patients diagnosed with thyroid adenocarcinoma. A periclavicular lymph node was excised during lymphadenectomy surgery from a female thyroid cancer patient and lymphocytes were isolated and fused with MFP-2, generating tetroma cells.

The resulting hybridomas (tetromas) were tested for production of human antibodies reactive against thyroglobulin using an enzyme linked immunoassay (ELISA) procedure. Purified human thyroglobulin was used to coat a microtitre plate. Results are shown in Figure 6. Thirty-three of 144 tetromas exhibited a response against the thyroglobulin antigen. Eight of these were particularly strong. (See Figure 6). Thus, lymph node-derived tetromas from this thyroid cancer patient were producing anti-thyroglobulin antibodies. This was an

unexpected and surprising result because the patient had no known history of autoimmune (i.e. anti-thyroid antibodies) disease. This suggests that the antibodies produced in this patient to thyroglobulin were induced by the presence of cancerous thyroid adenocarcinoma cells. Cancerous thyroid adenocarcinoma cells are known to secrete thyroglobulin. This experiment demonstrates that tumor cells can induce a humoral immune response to tumor-associated antigens and that the antibody-producing cells can be identified and immortalized through the techniques described herein using the MFP-2 fusion partner in order to produce human anti-tumor monoclonal antibodies.

Production of human monoclonal antibodies against breast cancer associated antigens. In another experiment, human monoclonal antibodies were produced against cancer associated antigens using lymph node and peripheral blood lymphocytes from breast cancer patients. Axillary lymph nodes were excised from breast cancer patients who underwent mastectomy or lumpectomy. Lymphocytes isolated from these lymph nodes were fused to MFP-2 and the resulting tetromas were screened against breast cancer cell lines MCF7, SK-BR-3, ZR-75-1. Nearly all the tetromas were producing IgG or IgM (approximately 85% and 10% respectively). Surprisingly, nearly 15% of the tetromas assayed against breast cancer cell lines produced antibodies specifically directed against cancer cells. The tetroma supernatants were tested in two ways: (1) on a live cells in the CELISA (cellular ELISA) assay and (2) by Western blotting using cell lysates. The molecular weight range of the specific antigens recognized by human monoclonal antibodies was 25 to 160 kDA. In order to delineate the nature of the antigenic target, immunoprecipitation followed by microsequencing is performed. In addition, random peptide combinatorial libraries are used to identify the molecular targets of the cancer-specific antibodies.

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In one patient with Stage IV breast cancer, lymph nodes were not available so PBLs were fused to MFP-2 and 156 tetromas were obtained. The tetromas were analyzed for immunoglobulin production as well as for cancer-specific antibody production. IgM was produced by 28 tetromas; 87 tetromas produced IgG. Four of the IgM antibodies and seven of the IgG antibodies were identified as reactive against cellular antigens; three IgM anti-bodies and four IgG antibodies were specific for breast cancer cells. The rest of the tetromas exhibited immunoreactivity against other cell types including human prostate cancer cell lines, human diploid fibroblasts and human skin fibroblasts. These latter antibodies were probably directed to common antigens (common for normal and cancerous cells).

The PBLs were isolated from the blood of a patient who received 77 cycles of chemotherapy which would reasonably be expected to have a depressing effect on the patient's immune system. None-the-less, this patient still produced anti-cancer antibodies suitable for fusing with MFP-2.

Human tetromas generated from fusing MFP-2 and prostate cancer lymphocytes are tested for the presence of PSA-specific antibodies as well as antibodies directed to prostate cancer cell lines LNCaP, DU-145, and PC-3.

Production of human antibodies against infectious disease-associated antigens. Infectious diseases are commonly accompanied by a well-developed humoral and cellular immune response. Patients with certain infections often contain large numbers of specific antibody producing cells. One important application of the antibody immunotherapy described by the present invention, is the production of human monoclonal antibodies to proinflammatory cytokines which are involved in septic shock. Among these targets are cytokines such as tumor necrosis factor α (TNF- α) and interleukin-1a (IL-1a). Additional targets include other

cytokines and lymphokines, infectious agents and their toxins, including tetanus toxin, anthrax toxin, botulinum toxin, and lipid A. The peripheral blood of patients infected with bacteria, fungi, protozoa or viruses typically contains circulating antibody-producing cells which can be isolated and used as a source for fusion with MFP-2. For example, PBLs from patients with septic shock, Hanta virus infection, HIV, HTLV-I, HTLV-II, influenza, hepatitis, or herpes virus can be fused with MFP-2 and the resulting tetroma cells can be screened against the respective antigens. In AIDS, in particular, patient lymphocytes can be immortalized using the techniques described herein in order to generate bulk quantities of anti-HIV antibodies for use in passive immunotherapy in an autologous or heterologous manner.

Production of human antibodies against autoimmune disease.

A general consideration for the use of human monoclonal antibodies in autoimmune disease is to block autoantibodies, or to block CD4⁺ T cells which are involved in autoimmune cellular cytotoxicity. In one approach, human monoclonal antibodies against CD4⁺ cells are generated following fusion with the MFP-2 trioma cell. Resulting tetroma cells which produce anti-CD4 antibodies are used to reduce or deplete CD4⁺ T cells, thereby relieving autoimmune cellular attack. In another approach MFP-2 is used to generate tetroma cells capable of producing anti-idiotypic antibodies directed to specific autoantibodies. For example, autoimmune thyroiditis is an autoimmune dysfunction in which there is a high titer of anti-thyroglobulin antibodies in a patient's plasma. PBL-derived lymphocytes are isolated from such patients for fusion with MFP-2. The resultant tetroma cells are screened for those capable of producing antibodies with a substantial anti-idiotypic immune response directed against the autoantibodies reactive with thyroglobulin. These anti-idiotypic antibodies are then used to modulate the autoimmune disease by reducing or depleting the anti-

thyroglobulin antibodies. Such an approach may be used autologously or heterologously. In an autologous approach, the anti-idiotypic antibody-producing cells are identified in peripheral blood of the patient to be treated, then isolated and fused with MFP-2 and following selection for specific anti-anti-thyroglobulin antibodies, passively administered to the original patient. In a heterologous approach, the anti-anti-thyroglobulin antibodies are administered to a different patient.

Other Applications: Preventing rejection of transplanted organs, blood clotting. Among other applications of human monoclonal antibodies, is prevention of organ transplant rejection by blocking T cells through the OKT-3 (anti-CD3) marker. Antibodies to adhesion molecules (anti-integrin antibodies) also prevent migration of immune cells, which is important, for example in rheumatoid arthritis. Blood clotting may be modulated, for example, in acute cardiac ischemia following coronary angioplasty, using human monoclonal antibodies against GPIIb/IIIa of platelet. Intravenous infusion of immunoglobulins helps to neutralize the Fc-receptor mediated cell aggregation of platelet or other blood cells (e.g. thromobytopenic purpura).

In addition, this approach may be used to detoxify or neutralize toxin or venom exposure. Such exposures include, but are not limited to snake, spider or poison toad bites or yellow jacket or scorpion stings. The horse anti-serum currently used to neutralize rattle snake venom causes serum sickness disease in 30% of cases.

There is a shortage of natural human immunoglobulin required for these kinds of treatments. The human monoclonal antibody production system described herein facilitates production, in vitro, of unlimited quantities of human immunoglobulins which can be selected to fit particular need. For example, in the case of

immunoglobulin which blocks Fc receptors, instead of
treating the patient with the pooled preparation of
immunoglobulins where only a small fraction of molecules
possess the required qualities, the immunoglobulin
5 preparation of the molecules with the required properties
can be produced using the fusion partner described herein.

Discussion

There has long been a need for human monoclonal antibodies
10 for diagnosis, treatment, and monitoring of cancer.
Attempts to employ xenoantibodies in clinical trials have
not produced promising results. Non-human antibodies from
mice, for example, cause development of a human anti-mouse
immune response, sensitization to foreign protein which may
15 eventually result in anaphylactic reaction, and lack of
biological effect since the effector properties of the
xenoantibodies may mismatch the components of the human
immune system. Human monoclonal antibodies have numerous
advantages. One is that human monoclonal antibodies can
20 identify those tumor-associated antigens (TAA) which are
immunogenic only in humans, while xenoantibodies in most
cases recognize those antigens and antigenic epitopes which
express immunodominance in a host and are often the tissue
specific epitopes. Another advantage is the well-developed
25 interaction of human monoclonal antibodies with the
effector components (such as complement) of the host immune
system. In addition, allergic and/or anaphylactic reaction
to the injectible human monoclonal antibodies is less of a
concern since human monoclonal antibodies are syngenic in
30 human subjects. Alternative attempts have been made to
develop antibodies such as chimeric antibodies (partially
human, partially murine), where the Fc part of the murine
immunoglobulin was substituted with the human IgG-Fc.
Humanized antibodies, are human immunoglobulins grafted
35 with the CDR regions of the specific murine antibodies.
Single chain (Fc) human antibodies have been developed in
phage using phage display libraries. A downside of these
approaches is that the resulting antibodies are not

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natural; they have not emerged as part of a natural immune response to cancer or infectious agent.

5 Use of the hybridoma techniques described herein and the
availability of the MFP-2 trioma fusion partner cell line
described herein, facilitates identification,
immortalization, and ex-vivo expansion of antibody-
producing cells which emerge in vivo as a result of natural
humoral immune responses to an antigen. Since such cells
10 are a part of the natural immune system response, the
antibodies produced by these cells dovetail with the other
components of the immune system and are able to provide an
effective and specific biological response.

15 A number of breast cancer specific antigens have been
described which are potential targets for the immunotherapy
of cancer, including HER2/neu, Mucin 1 and Mucin 2, p53, c-
myc, blood antigens T, Tn and sialyl-Tn, tuncated form of
EGF, Lewis-Y antigen and others. The presence of
20 circulating antibodies to these antigens have also been
described in cancer patients. (G. Moller, 1995). Lymph
nodes are important sites of such antibody-producing cells.
By isolating lymph node (or peripheral blood) lymphocytes
and immortalizing them by fusing them with human hybridoma
25 fusion partner MFP-2, hybrids (tetromas), which produce
antibodies directed against cancer-associated antigens may
be obtained. As described above, specific monoclonal
antibody producing cells are identified and may be produced
in unrestricted fashion, ex-vivo (using bioreactors, SCID
30 mice, etc). The antibodies may be used therapeutically as
passive immunotherapy either autologously in the same
subject or heterologously in a different subject. Even
another cancer may be treated, provided there is an
overlapping tumor antigen.

35 Syngenic or allogenic use of human monoclonal antibody can
be highly effective since such an antibody can be infused
many times without the risk or threat of developing an

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anti-xenogenic immune response. The infused antibodies, depending on their effector functions, can initialize complement dependent cytotoxicity of the target tumor cells, or antibody-dependent cellular cytotoxicity antibody dependent cellular cytotoxicity (ADCC) (by NK or CTL cells), or provide direct cytotoxic effect through apoptosis.

Summary

A unique fusion partner cell line, MFP, was obtained which can be used to generate specific human monoclonal antibodies. These monoclonal antibodies may be in vivo based on a natural immune response to infectious agents, cancer cells or an autoimmune dysfunction, or can be in vitro based by immunization of human lymphoid cells in vitro.

The methods described herein for generating specific monoclonal antibodies may be used to provide adoptive humoral immunotherapy either as an autologous procedure or as a heterologous procedure. Lymphocytes isolated from a patient with a cancer or infectious disease are immortalized by fusion with MFP-2. The resulting tetromas, producing antibodies directed to the respective antigens, are selected in vitro. Following selection, these antibody-producing cells are expanded and antibodies may be produced using a bioreactor or immune-deficient mice (e.g., nude mice or SCID mice). Such antibodies may then be used for the treatment of the original donor as an autologous adoptive immunotherapy procedure or for the treatment of a different subject as a heterologous, adoptive immunology procedure.

The developed antibodies may also be applied both to invasive diagnostics (imaging, immunoscintigraphy) or therapy (drug targeting, radioimmunotherapy, complement-dependent cytotoxicity, ADCC, apoptotic cytotoxicity etc.)

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Experimental Procedures

Hybridoma fusion partner MFP-2 was developed as a trioma cell line by fusing non-producing heteromyeloma B6B11 with human lymphocytes isolated from the paracervical lymph node.

Isolation of lymphocytes. Paracervical lymph nodes from a patient diagnosed with metastatic thyroid cancer were excised during the surgery and placed into sterile conservation media RPMI1640 supplemented with L-glutamine (4mM), non essential amino acids (100X stock), vitamins (100X stock), sodium pyruvate (1mM) and Gentamicin (2x concentration). Lymph node tissue was transferred to a 100 mm tissue culture TC dish in the same media and gently disrupted with forceps and scissors. The disrupted tissue was passed through a metal sieve (50 mesh) using a glass pestle. The suspension was transferred into 15 ml sterile conical tubes containing lymphocyte separation media (Histopaque 1.077 Sigma) as an underlying layer at a ratio of 2:1 (lymphocytes suspension: Histopaque). Following centrifugation at 400 X g for 20 minutes, an opaque ring formed at the border between layers. Red blood cells (RBC) were present as a pellet at the bottom of the tube. If RBC are not present in the starting lymphocyte suspension (which is a quite normal situation for lymph nodes) the separation step can be skipped. The opaque ring containing lymphocytes was carefully collected using a Pasteur pipette and was diluted 10-fold diluted with regular serum-free RPMI 1640. Cells were spun at 300 X g for 10 minutes and washed twice with media.

The final lymphocyte suspension was diluted with media and cells were counted using 0.05% Trypan Blue. Cell viability after isolation was usually 95%. Total yield was approximately 4×10^7 cells.

Preparation of B6B11. Heteromyeloma B6B11 was grown in RPMI 1640 with 10% cosmic calf serum (Hyclone), standard set of supplements (L-Glu, 4mM non-essential amino acids, vitamins, Sodium Pyruvate) without antibiotics. Before fusion, cells were cultured in the presence of 8-Ag (20 μ g/ml) to avoid reversion of HAT-sensitive cells to wildtype. Cells were grown to a density of 10% in logarithmic growth phase.

Cell fusion. Both B6B11 cells and lymph node lymphocytes were washed 3 times by centrifugation at 300 X g for 5 minutes in order to remove any residential protein in the media. Cells were mixed at a ratio of 5:1 (lymphocyte: myeloma) and spun at 300 X g for 10 minutes. The supernatant was carefully and completely removed the pellet was "puffed" gently and 100 μ l of PEG/DMSO solution warmed to room temperature was added to the cell mixture which was gently tapped for 3 minutes. Then 15 ml of Hank's Balanced Salt Solution (HBSS) and PBS (1:1) (from a 10x stock, Cellgro) were added as follows: 10 ml slowly in 10 minutes, then 5 ml over 5 minutes, then 10 ml of complete media (media for cell culturing) over 5 minutes and finally 5 ml over 1 minute. The total volume was 30 ml. Then 600 μ l of HT solution (of 10x stock) and 1 drop (about 20-30 μ l) of DMSO were added to the tube. The cell suspension was mixed in a tube, transferred to Petri dish (100x 15) and incubated in a 37°C CO₂ incubator overnight. The cells were then harvested, pelleted at 300 X g for 10 minutes and resuspended in complete media supplemented with HAT-solution and HT-solution (both from 50X stock) and then plated into 96-well plates in a 200 μ l volume at about 250,000 cells per well. Twice a week, 50% of the media was replaced with fresh media. Cells were cultured in the presence of HAT and HT for 14-20 days before screening for antibody production.

ELISA screening for nonspecific immunoglobulin. ELISA

plates were coated with polyclonal goat-anti-human IgG (Fc-specific) (Sigma), goat-anti-human IgM (μ -specific) (Sigma) or goat-anti-human Ig(G+M+A) H-chains (Sigma) in 100 μ l of plating buffer (0.1 M Sodium Carbonate, pH 9.0) at 100 ng per well. The plates were sealed with Parafilm or sealing covers and incubated overnight at 4°C. The antigen was washed out with distilled water twice. Residual drops of water were removed and 200 μ l of blocking solution (0.4% dry non-fat milk in PBS) was added to the wells. Complete cell culture media served as a negative control. Human serum (1:2000) was used as a positive control. Plates were incubated for 2 hours at room temperature or overnight at 4°C. The plates were washed 4 times with distilled water and secondary antibodies (same as capture antibodies but conjugated to HRP) diluted in 0.4% milk/PBS at 1:2000 were added to the wells. After 1 hour incubation at room temperature the wells were washed 4 times with H₂O and peroxidase substrate (ortophenylendiamine in phosphate-citrate buffer with peroxide) was added to the plates. The color reaction was stopped by adding 20 μ l of 10% sulfuric acid. Colorimetric reading was performed on a Multiscan reader at A₄₉₂. Samples which exhibited at least a 3-fold increase over background were considered to be immunoglobulin-producing cells.

Assay for the intracellular (non-secreted) presence of immunoglobulins or their individual chains. Cells which

did not secrete immunoglobulin in the supernatant culture media were tested for the presence of intracellular immunoglobulin-immunoreactive material. ELISA plates were coated with goat-anti-human kappa chain (Sigma), goat-anti-human lambda chain (Sigma) and goat-anti-human IgH (G,M,A) as described above. Cells were grown in 75 cm² flasks to the density 10⁶ cells per ml, harvested and washed 3 times with HBSS. Cells were resuspended in PBS and disrupted by sonication (8 x 15 seconds at 25 MHz on ice). The

suspension was spun for 15 minutes at 10,000 X g and the supernatant was used for immunoglobulin testing. An equivalent of 2×10^6 cells was used. As a negative control mouse fibroblasts 3T3 were used at the same protein amount equivalent. The rest of the protocol was the same as described above for the hybridoma supernatant testing. Clones which showed the signal equal to the control cells or lower were chosen as potential candidates for fusion with human peripheral blood lymphocytes. These trioma clones were designated as modified fusion partner series (MFP-S) and numbered sequentially (MFP-1, MFP-2, MFP-3, etc.) Six non-producing, non-secreting triomas were selected for further analysis.

Selection for 8-Ag resistant MFP mutants. To use MFP trioma cells as fusion partners, the MFP cells were placed in complete media containing an increasing amounts of 8-Ag. Resistance to 8-Ag is determined by the impaired enzyme HGPRT or its absence. Selection was therefore focused on cells which survived in the presence of 8-Ag. After 5 to 10 passages at the lower concentrations of 8-Ag ($5 \mu\text{g/ml}$) the survivors were cultured in media with a higher concentration ($10 \mu\text{g/ml}$). This was repeated until a concentration of $20 \mu\text{g/ml}$ was reached. After 5-6 passages in the presence of 8-Ag ($20 \mu\text{g/ml}$) cells were tested for their viability in HAT-media. None of the cells grown on 8-Ag survived after 3 days of culture in the presence of HAT.

Fusion efficiency. The MFP clones were tested for ability to fuse with lymph node lymphocytes and PBL. MFP-2 yielded approximately 2-3 hybrids per 10^5 lymph node lymphocytes and 0.7-1.5 hybrids per 10^5 of PBL. The immunoglobulin secretion rate for the hybrids developed using MFP-2 ranged between 0.5 to $15 \mu\text{g/ml}$ with no decrease over 7 months.

SECOND SERIES OF EXPERIMENTS

1. The trioma cell line MFP-2 used for fusion with human peripheral blood B-lymphocytes and human lymph node B-lymphocytes can be also used for fusion with human peripheral blood and lymph node T-cells and yield stable hybrids.
2. The trioma cell line MFP-2 can be used for fusion with peripheral blood and lymph node lymphocytes from two primate species: rhesus monkey (*Macaque mulatta*) and baboon (*Papio hamadryas*) yielding monkey immunoglobulin-producing hybrids. This has a potential application for the development of monkey monoclonal antibodies to different infectious agents to test them in primate models.
3. Trioma fusion partner cell line MFP-2 was adapted to the growth in protein-free media with the growth characteristics not different from those when cultured in serum containing or serum-free (protein supplemented-media).
4. It was inferred that, since MFP-2 can be cultured in protein-free media, the deriving hybridomas would be relatively easy to adapt to the same protein-free media.
5. Four out of 6 hybridomas were successfully adapted to protein-free media without changing the growth characteristics and loosing the antibody production. This feature of MFP-2 adds to the advantage of this cell line in developing hybridomas capable of growing in protein-free media.
6. 27 human hybridomas, producing human monoclonal antibodies to breast and prostate-associated antigens have been developed using MFP-2 and peripheral blood

and lymph node B-lymphocytes from breast and prostate cancer patients.

5 7. 23 human hybridomas derive from breast cancer patient and 4 derive from prostate cancer patients.

8. Prostate cancer-derived hybridomas:

10 1. hyridoma (32-B8) produces IgM, lambda antibody which reacts specifically with 2 human prostate adenocarcinoma cell lines and with one human breast adenocarcinoma cell line and is directed to an unknown antigen most likely of a non-protein nature (western blot is negative, although it well may be that the antigen is a protein but the antigen determinant is conformational and labile)

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20 2. hybridoma (32-F6) also produces IgM, lambda antibody reactive with both prostate and breast adenocarcinoma cells and recognizing the proteinous antigen of 60-kDa molecular weight.

25 3. hybridoma (39-A7) is also IgM, lambda antibody directed to an unknown protein target specific for both breast and prostate adenocarcinoma.

30 4. hybridoma (50-1B3) produces IgM, kappa antibody directed to both breast and prostate adenocarcinoma to a molecular target of unknown nature

9. Breast cancer-associated hybridomas are the following:

35 1. hybridoma (13-42), IgM, kappa recognizes protein antigen of ~42 kDa molecular weight which is present both on the surface and intracellularly of

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adenocarcinoma cells (breast and prostate) but not in human normal fibroblasts.

2. hyridoma (13-74), IgM, kappa reacts with protein antigen of ~65 kDa specific for the breast adenocarcinoma cells and expressed on the cell surface as well as intracellularly

3. hybridoma (13-82), IgM, kappa is reactive with intracellular protein antigen specific only for breast and prostate adenocarcinoma cells but not for human skin fibroblasts.

4. hybridoma (13-2C1), IgM kappa is reactive with a protein of ~100 kDa which is present both in adenocarcinoma and normal fibroblast cells.

5. hybridoma (22-3E9) isotype is not determined, recognizes several protein targets (which may be all related) of molecular weight 35, 45 and 250 kDa which are present on both adenocarcinoma and fibroblasts. The antigen is mostly on the surface of the cells. Reacts specifically with primary cancerous lesions

6. hybridoma (22-6E7), IgM, lambda, the antigen is unknown, the antibody is reactive only with breast adenocarcinoma cells in culture.

7. hybridoma (22-8D11), IgM, lambda, antigen is unknown, reacts with human breast and prostate adenocarcinoma cells in culture.

8. hybridoma (27-F7), IgM, kappa, reacts only with breast adenocarcinoma cells in culture. The antigen is a TAX interacting protein 2 of molecular weight ~35-40 kDa

9. hybridoma (27-B1) same as 27-F7, shows high specific reactivity with the cancerous lesions in primary tumors, no cross-reactivity with the connective tissue or with normal mammary epithelial cells
10. hybridoma (36-G7) antibody isotype is not determined; specificity is the same as 27-B1
11. hybridoma (27-F10), IgG, lambda, reactive with the protein approx. 200 kDa on breast adenocarcinoma cells
12. hybridoma (33-2F10), IgM, kappa, antigen is not known, reactive with breast adenocarcinoma cells
13. hybridoma (33-2H6), IgM, lambda, recognizes 65 kDa protein on breast and prostate adenocarcinoma cells but not on human skin fibroblasts
14. hybridoma (59-3G7), IgM, lambda, is reactive with a 70 kDa protein lamin A or C in adenocarcinoma cells. Cross-reactivity with other cells has not been Tested
15. hybridoma (59-2F6), IgG, lambda, reacts only with breast adenocarcinoma cells with unknown antigen
16. hybridoma (69-C12), IgM, kappa, reactive mostly with breast adenocarcinoma cells directed to a protein, 50 kDa 17 - hybridoma (76-2F6), IgM, lambda, reactive with unknown antigen only on breast adenocarcinoma cells
18. hybridoma (83-3A6), isotype not determined, reactive only with breast adenocarcinoma cells

19. hyridoma (85-E1), IgM, lambda, reactive only with breast adenocarcinoma cells expressing Her2/neu; antigen is not identified yet

5 20. hybridoma (88-1D8), isotype is not determined yet, recognizes protein antigens on breast cancer cells; molecular weights vary -70, 90 and 100 kDa

10 21. hybridoma (89) isotype is not determined, reactive only with Her2/neu- negative adenocarcinoma cells; antigen is not known

15 22. hybridoma (100-1F4), IgM, kappa, only reactive with breast adenocarcinoma cells; antigen is not known

23. hybridoma (100-2H3) similar to 100-1F4

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THIRD SERIES OF EXPERIMENTS

EXAMPLE I: Development of Fully Human Monoclonal Antibodies

5 **Introduction**

10 The present invention comprises a unique fusion partner cell line that fuses with human lymphocytes derived from lymph nodes, spleen, tonsils, or peripheral blood. The resulting hybrids have proved to be stable producers of human immune substances called immunoglobulins and represent a reliable source of human antibodies for immunotherapy. Using this fusion partner cell line, which was designated as MFP-2, we have developed several
15 monoclonal antibodies with specific reactivity towards human breast and prostate cancer.

Results

20 **Hybridoma Technology**

25 Fully human monoclonal antibodies (fhMAb) were developed through hybridoma technology using proprietary fusion partner cell line MFP-2 and human lymph node lymphocytes (LNL) isolated from the lymph node of Stage IV breast cancer female patient who underwent mastectomy and lymphadenectomy. Fusion of MFP-2 to LNL yielded several clones producing antibodies specifically reactive with established breast cancer cell lines SK-BR-3, MCF-7 and ZR-75-1. Two of the antibodies designated as 27.F7 and
30 27.B1 reacted specifically with the protein target from these cells of molecular weight approximately 43 kD, as was shown by Western blotting analysis of those cells' lysates both under reduced and non-reduced conditions. The hybridoma cell lines were adapted to growth in serum free
35 media reaching the density 1.5×10^6 cells per ml in flasks/TC dishes at the plateau phase. The cell line 27.F7 was also capable of growing in hollow-fiber Bioreactor reaching the

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density of $20-25 \times 10^6$ /ml and the cell line 27.B1 was growing effectively in spinner flasks. The production of the antibodies was 17 ug/ml/ 10^6 cells/24 h for 27.F7 and 49 ug/ml/ 10^6 cells/24 h for 27.B1. Both antibodies were IgM, k. For further studies of the molecular target for these antibodies, cells were cultured in quantities using serum free media and purification was done using size-exclusion chromatography of SephacrylTMS-200 (High Resolution) were IgM appeared in a void volume.

EXAMPLE II - Antibody Binding to Cancer Cell Lines

The antibodies produced reacted both with the human cancer cell lines and with primary tumor tissues. Antigen targets were identified for some of these antibodies. Two antibodies, 27.F7 and 27.B1, were directed to the same antigen, which was identified as Tax interacting protein, clone 2 (TIP-2). The antibodies 27.B1 and 27.F7 were reactive with three human breast cancer cell lines, MCF-7, SK-BR-3 and ZR-1-75, have tracer or no reactivity with human prostate cancer cell and negative with human fibroblasts.

Results

Elisa Assay

Cellular ELISA assay demonstrated the binding of 27.F7 and 27.B1 to human breast cancer cell lines in a specific manner, and no binding to human skin or trunk fibroblasts.

Flow Cytometry

Flow cytometry studies revealed that the antigen target is accessible on the surface of live cells as well as in cytosol of formaldehyde-fixed cells. However, the pattern of antibody binding to the cells was different, indicating that these antibodies probably recognize different epitopes of one and the same antigen. Antibody 27.B1 reacted with

the surface of breast cancer cells SK-BR-3 and MCF-7 and did not react with live prostate cancer cells PC-3 and LNCaP and with live human fibroblasts (Fig.7). However, when formaldehyde-fixed cells were used in flow cytometry analysis it showed that 27.B1 antibody reacted with both breast cancer cell lines and with prostate cancer cells LNCaP, although it was still negative to human fibroblasts. Antibody 27.F7 showed a different pattern of reactivity: it reacted with the fixed primary fibroblasts, apparently with some intracellular epitope. Using cell lysates prepared from three breast cancer cell lines (SK-BR-3, MCF-7 and ZR-75-1), three prostate cancer cell lines (LNCaP, PC-3 and Du-145) and two human fibroblast cell lines (Hs556.Sk and Hs143.We)

Western Blot

The Western blot analysis demonstrated that both antibodies 27.F7 and 27.B1 react with the protein of approximately 43 kD which appears on a blot as a double band. This protein is profoundly expressed in all three breast cancer cell lines, not expressed in two human fibroblast cell lines and very weakly in prostate cancer cells PC-3 and Du-145. LNCaP cells show expresses negligible if any level of this protein (Fig.8).

Immunocyto- and Histochemical Studies

Immunocyto- and histochemistry studies using established human cell lines and primary and metastatic lesions of tumor tissues from a number of breast and prostate cancer patients showed a very specific pattern of immunostaining of breast and prostate cancer cells (Fig. 9), primary tumors (Fig. 10, 11, 12 and 13) and metastatic lesions in the lymph nodes (Fig. 14). Both fixed and freshly frozen tumor tissues were positive when immunostained with antibodies 27.B1 and 27.F7 (Fig. 15). Out of 10 breast cancer cases tested in immunohistochemistry with fhMAb 27.B1 all 10 were positive while the matching number of

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EXAMPLE III - Identification of the Antigen

Based on the antibodies described above, a novel tumor associated antigen specific to breast and prostate adenocarcinoma has been identified as GIPC (Tax Interacting Protein 2). The method used to identify this novel tumor-associated antigen was SEREX (Serological analysis of antigens by Recombinant Expression cloning or spontaneous antibody responses to tumor-associated antigens) (Fig. 20). This method was originally developed in the Ludwig Institute for the purpose of identifying specific protein targets for the antibodies found in plasma or serum of cancer patients(1). The invention describes a 43-kDa protein, which belongs to so-called PDZ domain containing proteins. PDZ domains are protein motifs of 80-100 aminoacids where the repeat consensus of GLGF is a distinctive characteristic. The PDZ domain (named after mammalian postsynaptic density protein PSD-95, *Drosophila* disc large protein Dlg and a mammalian tight junction protein ZO-1) is found in more than 50 proteins, which for the most part appear, unrelated to one another. These proteins are commonly involved in signaling networks, such as G protein-mediated signaling pathways. PDZ domains are found, for example, in signaling molecules such as Dlg, nitric oxide synthase (NOS), protein-tyrosine phosphatase, membrane-associated guanylate kinases (MAGUK), and so on.

Most PDZ domain-containing proteins are associated with the cytoskeleton and apparently involved with formation of multimeric protein complexes (2,3). The only PDZ domain-containing protein associated with human colon cancer was described by Scanlan et al. (4,5). This antigen, NY-Co-38/PDZ-73, was identified through IgG autoantibodies developed in colon cancer patients. The same authors also described a few tissue-specific isoforms of PDZ-73, that appear to be truncated forms containing one or two PDZ domains (the original PDZ-73 form has three domains). The

function of these proteins is not known, although they bear the structural similarity with the MAGUK family of proteins. The PDZ domain, although its particular function is not clear, is believed to participate in protein-protein interaction and formation of large protein networks.

TIP-2 was recently identified by Rousset et al. (1) as one of 6 cellular proteins of unknown function that interact with the C-terminus of Tax oncoprotein through their PDZ domain. As C-terminal motif S/TXV is important for interaction with PDZ domain, it turned that Tax oncoprotein preserves interaction with TIP-2 even if the critical C-terminal valine is replaced, for example, with alanine, while all other Tax-binding PDZ domain-containing proteins lose their binding potential.

Results

TIP-2 was identified by screening breast cancer patients' B-cell-derived antibodies on a cDNA expression library prepared from human breast cancer cell line SK-BR-3. Briefly, poly(A)+ RNA was isolated from the cells, transcribed into cDNA and ligated into lambda pseudolytic phage, resulting in approximately 5×10^5 recombinants. The phage was amplified in *E. coli* Y1090 and then transferred to nitrocellulose membranes, which were treated with human antibodies. After exposure to antibodies the membranes were treated with anti-uchain rabbit polyclonal antibodies conjugated to horseradish peroxidase. Positive cDNA clones were converted into plasmid forms by excision *in vivo*, and the plasmid DNA was purified and submitted to sequence analysis. The resulting sequence was submitted to homology search using a Gene Bank database. Two human monoclonal antibodies (27.F7 and 27.B1) developed from breast cancer patient's lymph node B-cells were identified as antibodies reactive with TIP-2--however apparently with different epitopes.

One of the antibodies, 27.F7, was produced in a Bioreactor in large quantities and used for immunoprecipitation of TIP-2 from the SK-BR-3 cell lysate. The precipitate yielded 2 bands of molecular weight characteristic of TIP-2 and corresponding to the bands recognized by anti-TIP-2 antibodies in Western blotting of cell lysates. The nitrocellulose membrane strip containing bands of TIP-2 was implanted subcutaneously into Balb/C mice in order to immunize them. After two implantations the mice developed a significant immune response to TIP-2 as proved by Western blot analysis of mice sera against SK-BR-3 cell lysates (Fig. 21 and 28). The immune serum from these mice was positive in immunohistochemistry of actual tumor tissues (Fig. 23). These mice will be used for further development of mouse anti-TIP-2 monoclonal antibodies.

Using fhMAb 27.F7 an estimate of its affinity and also of number of TIP-2 molecules on the surface of SK-BR-3 was made. It was found that there are two subsets of TIP-2 molecules (which corresponds to Western blot data) which have different affinity to 27.F7. One subset (isoform) of TIP-2 is present at about 60 000 copies per cell and binds 27.F7 with the $K_a=4.2 \times 10^{11} M^{-1}$ and another one is present at 230 000 copies per cell with the $K_a=3.3 \times 10^9 M^{-1}$ (Fig. 24). Western blot analysis using human breast cancer cell lysates as well as primary tumor lysates showed a strong expression of TIP-2 in all tumor lesions and no traces of this antigen in normal unaffected breast epithelia (Fig. 25) These data were consistent with immunohistochemistry studies of the tissue section from the same clinical cases (data not shown).

Coupling 27.F7 to Liposomes

In order to explore the possibility of using anti-TIP-2 antibody as a vector for liposome delivery, a few different methods of coupling 27.F7 to liposomes were tested. Given the fact that the antibodies were of IgM, k isotype problems with the chemistry of coupling IgM to liposomes

were expected. One of the protocols proved to be most effective yielding high ratio of antibody coupling to liposomes and preserving the antibody intact and reactive to TIP-2 as has been demonstrated by Western blot (Fig. 26).

TIP-2 Identification in Breast Cancer Patients

Also attempted were experiments to identify TIP-2 in serum or plasma of breast cancer patients. The rationale for such an assumption is that since TIP-2 is expressed on the surface of the cells, some part of it can be shed into circulation or even if this is not a case, then it still may appear in advanced stage disease patients' sera as a result of necrosis of the tumor or as a result of chemotherapeutic treatment. Since there is no ELISA assay for such a testing, patients' sera was tested for TIP-2 using Western blot of the whole serum sample and fhMAb 27.F7 as a tag. This method did not work because of a technical problem: The abundance of human serum albumin (HSA) in human serum masks the region on a gel where one would expect to locate TIP-2. Spiking the serum sample with the SK-BR-3 cell lysate (containing TIP-2) showed that TIP-2 could be identified both in human serum and human plasma by Western blot. In order to make the identification of TIP-2 in serum more profound a stepwise alcohol fractionation of human serum spiked with SK-BR-3 cell lysate was done to identify the alcohol concentration sufficient to precipitate TIP-2. It was shown (Fig. 27) that TIP-2 can be completely precipitated by 10% alcohol, while HSA and immunoglobulins (the major protein constituent of human serum) were still remaining in a solution. This can make the identification of TIP-2 in serum using Western blot easier. A two site immunoenzymatic assay, using high affinity mouse antibodies would provide another means of TIP-2 antigen identification.

Discussion

One of the targets which appeared is the PDZ domain containing protein localized both in cytosol and cell membrane of human breast cancer cells. This protein, called GIPC or TIP-2 (Tax interacting protein clone 2), is involved in vesicle trafficking and formation of protein networks. It has several properties, such as the ability to bind to RGS-Ga interacting protein, C domain, binding to HTLV-1 oncogene tax and bonding both to α -actinin and glucose transporter 1. While the precise physiological role of this protein is not known, it shows a consistent overexpression in breast cancer cells, with negligible if any expression in prostate cancer cells, and no expression in human fibroblasts. GIPC/TIP-2 is a 42kDa protein which is present on a Western blot in a form of a doublet, probably because it has two open reading frames in its N-terminus. The number of copies per SK-BR-3 human breast cancer cell is quite high, approximately 300,000 copies per cell. Two fully human antibodies through which this antigen was identified belong to IgM isotype and have different epitope specificity. One of the antibodies, 27.B1 has a significant immunoreactivity with the surface of TIP-2-positive cells, while another, 27.F7 reacts only with the fixed cells, i.e. intracellularly. 27.B1 also expresses the profound internalization ability, while 27.F7 does not. Testing 27.B1 for its biological effect in the presence and absence of complement revealed that this antibody can cause the cellular cytolytic/cytostatic effect without the complement. The mechanism of this effect is most likely an apoptosis.

The protein identified herein was recently described as GIPC (GAIP Interacting Protein, C terminus), a protein which binds through its the PDZ domain to the C-terminal motif of the target proteins (6). In this case the target protein is GAIP (G_{ai3} Interacting Protein), a membrane-anchored RGS (Regulators of G Signaling) protein,

that interacts with α_{13} subunit of G protein and enhances its GTP-ase activity, facilitating deactivation of the G protein (Fig. 18, 19)(7). GIPC is the only protein described to date that binds to the C terminus of GAIP. The functional meaning of this interaction is not known. Recently, Rousset et al. (8) isolated an incomplete GIPC cDNA using Tax transactivator protein from HTLV-1 as a bait. They called this form of GIPC TIP-2 for Tax Interacting Protein clone 2 and showed that this form effectively interacts with the C-terminus of Tax oncoprotein. Tax oncoprotein is not the only oncoprotein that binds to PDZ domain through its C-terminus. E6 oncoprotein of human papilloma virus (HPV) (9) and E4 oncoprotein of D adenovirus type 9 (Ad9) also have C terminal motifs that bind to the PDZ domain (10). Such binding could be an underlying mechanism in the development of HPV-associated cancers or as in the case of E4 oncoprotein of mammary tumors (Ad9 is unique in eliciting only estrogen-dependent mammary tumors in female rats [11]). For all three oncoproteins the C terminal region is crucial for eliciting transforming potential (8,9,10). As C-terminal motif S/TXV is important for interaction with PDZ domain; it turned that Tax oncoprotein preserves interaction with TIP-2 even if the critical C-terminal valine is replaced, for example, with alanine, while all other Tax-binding PDZ domain-containing proteins lose their binding potential. TIP-2 was identified by screening breast cancer patients' B-cell-derived antibodies on a cDNA expression library prepared from human breast cancer cell line SK-BR-3. Briefly, poly(A)+ RNA was isolated from the cells, transcribed into cDNA and ligated into lambda pseudolytic phage, resulting in approximately 5×10^5 recombinants. The phage was amplified in *E.coli* Y1090 and then transferred to nitrocellulose membranes, which were treated with human antibodies. After exposure to antibodies the membranes were treated with anti-u chain rabbit polyclonal antibodies conjugated to horseradish peroxidase. Positive cDNA clones were converted into plasmid forms by

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excision *in vivo*, and the plasmid DNA was purified and submitted to sequence analysis (Fig. 8). The resulting sequence was submitted to homology search using a Gene Bank database. Two human monoclonal antibodies (27.F7 and 27.B1) developed from breast cancer patient's lymph node B-cells were identified as antibodies reactive with TIP-2 -- however apparently with different epitopes.

The GeneBank/Protein Database information for this protein is the following: NCBI reference - NP005707.1PGGLUT1CBP; Homo sapiens RGS-GAIP interacting protein GIPC mRNA, complete cds (AF0889816); Homo sapiens Tax interacting protein 2 mRNA, partial cds (AF028824). The subject invention demonstrates that this antigen, **Tax Interacting Protein 2 (TIP-2)**, can serve as a distinctive and specific marker for breast and prostate adenocarcinoma.

Summary of Experiments

Using a specific fusion partner cell line MFP-2 were developed two fully human antibodies to breast and prostate cancer-associated antigens. Both antigens were reactive with a 42kDa-protein target, which was identified through SEREX technology as Ga-interacting protein, C terminus or Tax interacting protein, clone 2. This protein is specifically overexpressed in three human breast cancer cell lines, SK-BR-3, MCF-7 and ZR-1-75, has very low if any expression level in human prostate cancer lines, PC-3, LNCaP and DU-145 and no expression in two human fibroblast cell lines. The TIP-2 antigen was found to be expressed in all breast cancer tissues and most of prostate cancer. Normal breast epithelia were negative for staining with anti-TIP-2 antibodies as was benign prostate hyperplasia (BPH) tissue. Two fully human monoclonal antibodies against GIPC/TIP-2 antigen were directed against different epitopes and gave a distinctive pattern of immunoreactivity with human breast cancer cells. Antibody 27.F7 was

reactive both with formalin-fixed and live cancer cells SK-BR-3 and MCF-7, while antibody 27.B1 reacted with live and fixed SK-BR-3 cells and only with fixed MCF-7 cells. On the other hand antibody 27.B1 showed a rapid internalization, while 27.F7 would not internalize. Also, when tested for cytolytic/cytostatic effect in the presence and without complement, it appeared, that 27.F7 does not cause any cytotoxic effect on the cells, while 27.B1 causes cytotoxic effect which is not dependent on complement. The Scatchard analysis of number of copies of GIPC/TIP-2 antigen per cell showed that thus antigen is present at quite high number of copies reaching somewhat 300 000 copies per cell. This includes the total number of TIP-2 molecules, both on the surface and in cytosol. Using one of the human antibodies, 27.F7 as immunoprecipitation bait, isolated was a small amount of TIP-2 and were able to raise several mouse monoclonal antibodies to this antigen. All the antibodies react in Western Blot with the protein band, which corresponds to TIP-2, and also give distinctive and specific positive staining of cancer cell and primary tumor tissues. Using human antibodies it was shown that normally GIPC/TIP-2 is not secreted or shed by cancer cells but can be found in culture media only as a result of cell destruction. The treatment of SK-BR-3 cells with the increasing amounts of Taxol, showed TIP-2 antigen released into the media in a dose dependent manner, therefore indicating that this marker is valuable for the monitoring of natural or chemotherapy-induced necrosis of tumor lesions.

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FOURTH SERIES OF EXPERIMENTS

Protein Antigens Identified by Natural Human Monoclonal
Antibodies Developed from Breast and Prostate Cancer
Patients' B-Cells

INTRODUCTION

In addition to GIPC/TIP-2, the method described in the
third series of experiments (above) may be used to identify
other protein antigens, including those listed below.

EXAMPLE I: Human mRNA for KIAA0338 gene, partial cds

Fully human monoclonal antibody (fhMAb) 13.42 recognizes
the unknown antigen human mRNA of which is known for the
gene called KIAA0338 (sequence shown in Fig. 32). The
calculated molecular weight (MW) for this breast cancer-
associated marker is 103.5 kDa, although on Western blot it
shows the protein of molecular weight ~40kDa. Three MHC I
binding peptides were deduced from the sequence; these
peptides may be considered as peptide vaccine candidates.

EXAMPLE II: Human Non-muscle alpha-actinin mRNA, complete
cds; Homo sapiens actinin, alpha 4 (ACTN4) mRNA

fhMAb 13.2C1 recognizes non-muscle alpha-actinin of MW 105
kDa (sequence shown in Fig. 33) which is found in many
human tissues, but there are reports on the association of
this marker with breast cancer. We have deduced three MHC
I-restricted peptides, which can be considered as peptide
vaccine candidates for breast cancer. fhMAb 13.2C1 also
recognizes homo sapiens actinin, alpha 4 (ACTN4) mRNA
(sequence shown in Fig. 34).

EXAMPLE III: Human Clathrin Coat Assembly Protein 50 (AP50)
mRNA

fhMAb 22.8D11 is directed against breast and prostate
cancer-associated marker which is human clathrin coat
assembly protein 50 (AP50) of MW 50kDa. Although its mRNA
(sequence shown in Fig. 34) was reported in some human
tissues including ovarian tumors, the protein product seems
to be associated with breast and prostate cancer. To the
best of our knowledge this marker was not reported before
as being associated with these types of cancer. We have
deduced four MHC I -restricted peptides for their possible
significance as peptide vaccine candidates.

EXAMPLE IV: Homo sapiens gp 130 associated protein GAM
mRNA; Homo sapiens amino-terminal enhancer of split (AES)
mRNA; Antiquitin 1 mRNA

fhMAb 33.2H6 is directed against human gp130-associated
protein GAM of MW ~22kDa. This protein was never reported
before as breast cancer-associated antigen, although its
mRNA (sequence shown in Fig. 37) was found in ovarian
tumors. Its homologue human amino-terminal enhancer of
split (AES) mRNA (sequence shown in Fig. 38) has an unknown
function but has been proposed as a candidate human cancer
antigen. We have deduced one MHC I binding peptide as
possible peptide vaccine candidate. The same antibody was
reactive towards antiquitin 1 (MW ~55 kDa)--26g turgor
protein homolog (sequence shown in Fig. 39). Partial mRNA
for this antigen was found in a number of human tissues,
however it was never reported before for its association
with breast cancer. We have deduced three MHC I-restricted
peptides from the amino acid sequence of this protein.

EXAMPLE V: ARP2/3 Protein Complex 41 KD subunit (P41-ARC),
mRNA

fhMAb 39.A7 is directed against ARP2/3 protein complex 41 kDa subunit (P41-ARC). This protein was not known for being associated with breast cancer before. We have deduced one MHC I-restricted peptide as a candidate for peptide-based vaccine (sequence shown in Figure 40).

EXAMPLE VI: Homo sapiens seb4D mRNA; Homo sapiens seb4B mRNA

fhMAb 50.1B3 recognizes the protein in breast and prostate cancer tissues which was identified as seb4B/4D antigen of MW~ 25kDa. This protein also was not known for its specific association with breast cancer. The function is unknown, while its mRNA was found in a number of normal human tissues. We have deduced two MHC I-restricted peptides from the primary sequence of this protein (sequences shown in Figs. 41a and 41b).

EXAMPLE VII: Homo sapiens lamin A/C (LMNA) mRNA

fhMAb 59.3G7 is reactive to human lamin A/C an intermediate filament protein, mRNA for which was found in many human tissues. The MW for this protein is~65 kDa. This protein was identified earlier by different research group through the serum antibody found in cancer patients. It is considered to be overexpressed in breast adenocarcinomas as well as in some other types of cancer. We have deduced three MHC I-restricted as potential candidates for peptide-based vaccine (sequence shown in Figure 42).